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EXPERIMENTAL VIRAL INFECTIONS IN THE EMBRYO AND FETUS
PRELIMINARY NOTES ON PATHOLOGIC FINDINGS WITH VIRUSES OF PSITTACOSIS, ECTROMELIA, AND RABIES *

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That various agents, especially microorganisms, are transmitted by the mother to the embryo is generally known. It is possible to prove the presence of microorganisms in the fetus and in the newborn and often to recognize the results of microorganismal activity in the form of a granuloma. Some of these organisms pass easily through the placenta, for example, *Treponema pallidum;* others, such as the tubercle bacillus, are transmitted only seldom. The circumstances which make such passage possible in one case and which prevent it in another case are not easy to understand. The placenta constitutes an essential, but also very complex, filter.

In studying such transmission, the well known infections in the newborn must be disregarded, even though post-mortem examinations of the newborn have revealed reactions. Inflammatory changes in the cardiac valves and in the myocardium are found, and from such reactions it is easy to receive the impression that infection must have taken place in the fetus during pregnancy. But this cannot be proved. In general, women are not easily infected during a normal pregnancy. In some cases there are indications of pneumonia or angina, but the anamnesis is not conclusively specific. Such reactions are usually limited and seldom serious. It must be stressed, however, that systematic examination of all organs of a stillborn child or of a child who died shortly after birth often reveals reactions in the lungs, myocardium, liver, and other organs, and these do point to inflammation *in utero*. These inflammatory changes have been described by Sorba (1949).

It is even more difficult to understand the reactions of the fetus in cases in which the mother is infected by German measles during preg-

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nancy (Gregg, 1941). In such cases, none of the reactions could be termed inflammation in the ordinary sense of the word; these changes are something entirely new. Here one finds malformations of the heart (even complete absence of some of its parts); of the eyes, including cataracts; of the head and teeth; of the ears, particularly the organ of Corti; of the intestines and other organs. Other types of anomalies have been manifested in unusual pigmentation (Werthemann, 1948) and in hypoplasia; for example, affecting the teeth. It is difficult to conceive that such "malformations" result from an inflammatory process. Above all, the organs of ectodermal origin are likely to suffer damage. In the ear, the organ of Corti is transformed and differentiation of the perceptive epithelium is defective.

Here I would point out that the expression malformation, as Töndury (1951) has already shown, is not really appropriate. Töndury proved, by examination of embryos, that destructive secondary growth and disturbance of differentiation appear in the lens of the eye and in the organ of Corti. These he assumed to be the result of an infectious process. The attendant circumstances are exceedingly complex. In the eye, growth was retarded, but the organs were otherwise normally developed. The fibers of the lens had degenerated and later dissolved. The embryonic fibers of the lens were the site of an especially intensive proliferation and had a tendency to grow. The lens is the site of very intense metabolic activity and it is probably for this reason that a virus infection in this organ sets up such a strong reaction. To describe these changes, the expression embryopathy is used.

Embryopathy can be caused not only by German measles, but by measles and by herpes zoster.

It can be safely stated that embryonic tissue reacts to a virus infection quite differently from adult tissue. Histopathologic reactions known to take place in many virus infections in adults cannot be proved to exist in the same form in the embryo or in the fetus. Probably a variety of factors are responsible for this. One cause is undoubtedly the increased metabolism of the embryonic cell. The circumstances in virus infections are probably quite similar to those in bacterial infections. In syphilis, for example, the tissues of the adult react quite differently to the invasion by the spirochete than do the tissues of the fetus. In the newborn child, syphilis commonly takes the form of inflammation of the lungs, of unusual skin diseases, or of osteomyelitis, but in syphilis of the adult these disease states are only rarely encountered.

During a recent visit to the Hooper Foundation, an opportunity

was afforded to undertake a preliminary investigation of the nature of the tissue reaction of the embryo or fetus provoked by infection of the mother or of the egg with certain viruses. At the suggestion of Dr. K. F. Meyer, viral infections were chosen that lead to severe, fatal illnesses in the animals studied—psittacosis, ectromelia or mousepox, and rabies.

Methods

For psittacosis, strains 6BC* and Borg (Louisiana); for ectromelia, the Hamsted strain†; and for rabies, the Flury‡ and Fox 16T strains were selected. The reactions of adult animals—the manner and site of infection and the structural changes—to infections with these strains are well known.

Mice in ascending stages of pregnancy were infected intranasally or intraperitoneally with either of the two psittacosis viruses (489 mice) in 20 per cent suspension of mouse spleen or yolk-sac material and in dilutions of 10^{-1} through 10^{-6}. Mouse spleen suspensions of ectromelia virus (535 mice) were inoculated intraperitoneally in a dilution of 10^{-6}. Some of the mice were sacrificed when infection was advanced (sixth to eighth day); others were examined after they had died of infection. Invariably the progress of infection in the mother mouse was determined by examination of impression preparations and search for elementary bodies or by subinoculation of tissue suspensions into mice. Portions of the liver, kidneys, spleen, and the entire uterus containing the embryos were fixed in picric acid (Bouin's fluid) and in mercuric chloride with formalin and trichloracetic acid (Susa fluid). Many of the fetuses and newborn animals, some of which were the products of spontaneous abortion, were similarly fixed and examined for lesions. Specimens were taken only from fetuses of typically infected mothers. In some series, 100 mice were infected at different stages of pregnancy, and 4 or 5 of these were sacrificed every day, in order to secure insight into the progress of the infection. This procedure assured a complete picture of lesions in the mother and correspondingly in the embryos and fetuses. Since the study was preliminary and exploratory and the primary concern was the nature of the reactions, no attempt was made to correlate the various stages of infection with the microscopic lesions. In subsequent studies special attention will be paid to this aspect.

The Flury strain of rabies virus (Koprowski and Cox, 1945) was

* Hooper Foundation strain, available since 1941.

† Original strain obtained through the courtesy of Dr. D. J. Davis, National Institutes of Health, Bethesda, Md.

‡ Original strain obtained through the courtesy of Dr. Hilary Koprowski, Lederle Laboratories, Pearl River, N.Y.

injected, undiluted as received, into the yolk sacs of eggs that had been incubated for from 7 to 12 days. On the eighth to the 16th days after inoculation, the embryos were dissected and fixed in the usual way. Some of the infected eggs were permitted to hatch and the young chicks were examined histologically. To learn whether rabies virus was present in the injected embryonated eggs, mice were inoculated intracerebrally with a suspension of egg material. The mice died, with symptoms of rabies, on the fifth to the tenth day. There were typical Negri bodies in sections of the mouse brains.

The Flury virus was also injected intracerebrally into pregnant mice. At the time of death, when there were Negri bodies in the central nervous system, the embryos were fixed and examined for anatomical changes.

A street rabies virus (Fox 16T) was similarly tested on pregnant mice; the mothers as a rule died on the fifth to the tenth day. Several embryos in different stages of development were examined in the same manner.

Results

The results of these studies vary considerably and it is not easy to interpret them. The vast number of cells in the embryo place considerable difficulties in the way of accurate examination. In many organs, particularly in the bones and the central nervous system, there is no maturity at birth. In these organs it is difficult to know what reactions have taken place in the cells. Degeneration and inflammation had apparently taken place simultaneously. In brief, the most important histopathologic changes can be said to have affected the liver, myocardium, spleen, and lymph nodes. Less extensive reactions were found in the lungs, the kidneys, and the brain. Examination of the mother animal, which was heavily infected by the virus, revealed, as a rule, other sites and types of degeneration and inflammation. This dissimilarity has been pointed out previously in spontaneous infections induced with the virus of equine abortion (Oppermann, 1938).

Instead of describing the characteristic findings at length, they are illustrated in Figures 1 to 17.

The typical lesions in the embryos and fetuses were, briefly, these: The myocardium after infection with psittacosis virus showed cellular infiltration. This was present in either a diffuse or granulomatous form. Psittacosis virus (Figs. 1 to 8), as well as that of ectromelia, induced hepatic necrosis with extensive hepatic cell degeneration and slight round-cell infiltration of the vessels. Some cellular reactions of the vessels had also taken place in the kidney and other organs. Minute

granulomas were often seen in the intima. With the naked eye, hypertrophy of the splenic pulp could be seen. Microscopically there were great numbers of reticular cells and the development of giant cells, as well as many atypical cells. In the lymph nodes, hyperplasia and desquamation of the sinusoidal endothelium were found; giant cells and numerous atypical elements were present also.

In the infection caused by ectromelia virus (Figs. 9 to 16), which Fenner (1949) investigated and discussed in a series of articles, there were, in general, severe histopathologic reactions in the mother animal. In my studies the liver tissue of the fetus sustained damage which often took the form of necrosis. The spleen was extensively necrotized and infiltrated by reticular cells. In the lymph nodes, the reticular cells were hyperplastic. The mucosa of the intestines was ulcerated and necrotic.

Rabies virus did not bring about essential histopathologic reactions in the artificial or cultural host—the chick embryo. After infection with the Flury strain, the embryo would develop normally without showing any signs of degeneration. No tissue reaction was found in the chicks that were allowed to hatch. It is surprising that there was no trace of genuine inflammatory processes in the brain (Fig. 17). However, the virus was present, because intracerebral inoculation of brain material from the chicken embryo into the white mouse was followed by typical viral encephalitis. After several days a great number of inclusion bodies were found in the brains of such white mice.

In summary, with certain virulent viral infections—ectromelia and psittacosis—produced experimentally in pregnant mice, it was possible to detect histologic evidences of reactions which can be regarded partly as genuine inflammation, partly as degeneration in the resulting embryos, the fetuses, and newborn animals. The chicken embryo remained unchanged by the rabies virus. With extremely virulent infections, the mouse embryo usually dies at an early stage of pregnancy. It is possible that the dead embryos are then dissolved. Such reactions are altogether dissimilar to those which take place in the mother animals.

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LEGENDS FOR FIGURES

FIG. 1. Psittacosis. Lung with dispersed groups of cells in the pulmonary tissue and increased infiltration of the interstitium. Fetus of a white mouse. × 170.

FIG. 2. Psittacosis. Myocardium with dispersed groups of cells along a coronary vessel. Fetus of a white mouse. × 85.

FIG. 3. Psittacosis. Liver with limited cushion-shaped granuloma of a vessel wall. Hyaline droplet degeneration of the cells. Fetus of a white mouse. × 160.

FIG. 4. Psittacosis. Fetus of a white mouse with eye in an early stage of growth and degeneration of the fibers of the lens. × 55.

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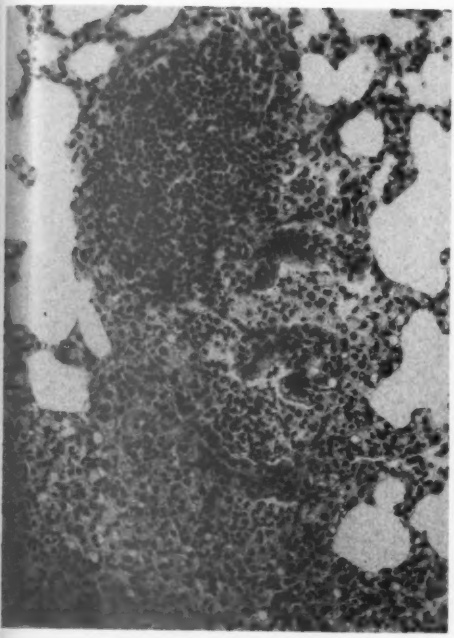

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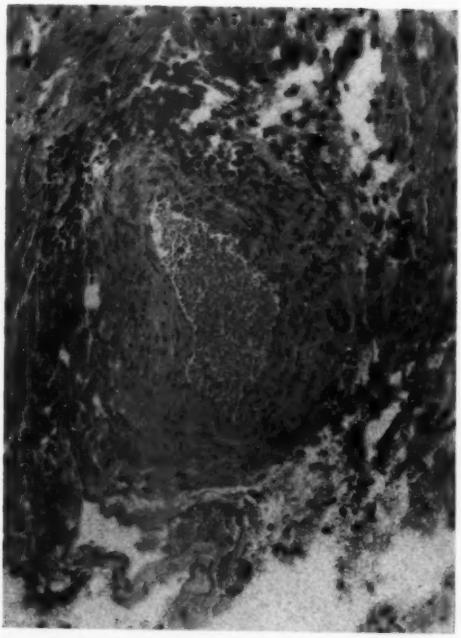

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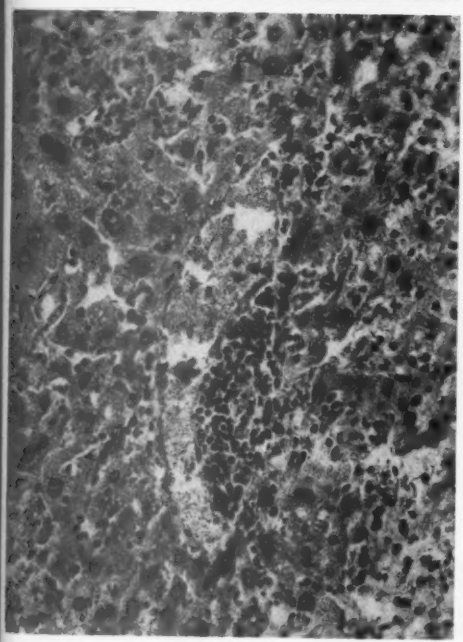

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FIG. 5. Psittacosis. Liver with recent degeneration of liver cells and slight inflammation. Fetus of a white mouse. × 385.

FIG. 6. Psittacosis. Liver with large necrotic region. Fetus of a guinea-pig. × 100.

FIG. 7. Psittacosis. Fetus of a white mouse with extensive degeneration of the liver and resulting cell débris.

FIG. 8. Psittacosis. Liver of a newly born white mouse, with inclusion body.

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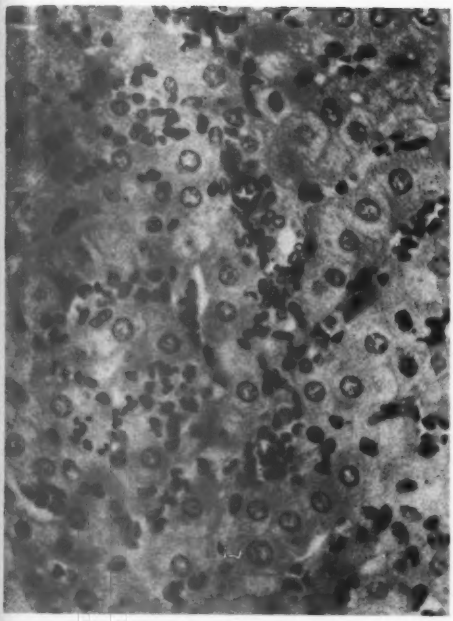

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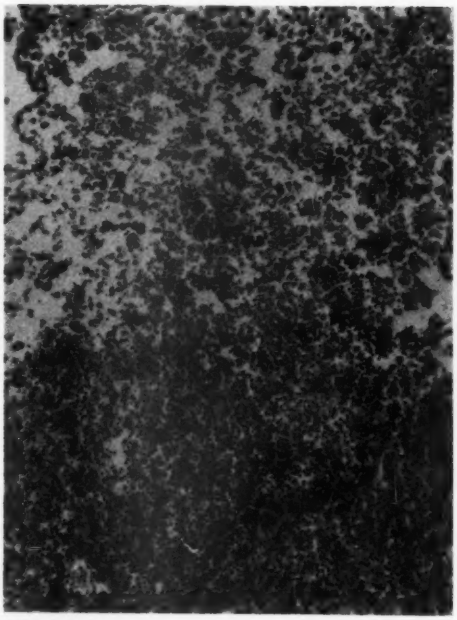

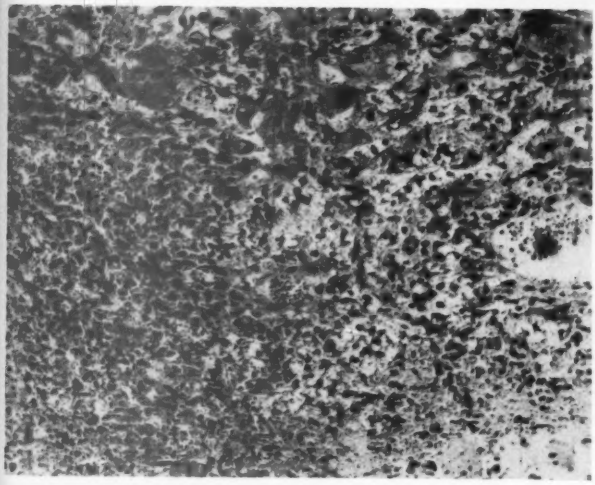

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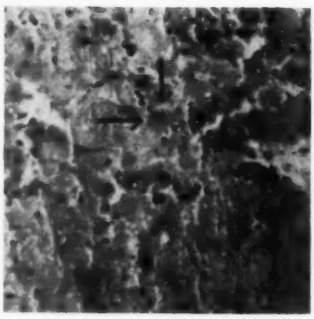

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FIG. 9. Ectromelia. Fetus of a white mouse with extensive necrosis of the liver. × 8.5.

FIG. 10. Ectromelia. Liver with limited areas of hyaline droplet degeneration, with partial dissolution of the nuclei. Fetus of a white mouse. × 315.

FIG. 11. Ectromelia. Liver with large necrotic areas with isolated clusters of living cells. Fetus of a white mouse. × 110.

FIG. 12. Ectromelia. Liver with atypical cells, with tumor-like elements (regeneration) and necrosis in an advanced stage. Fetus of a white mouse. × 260.

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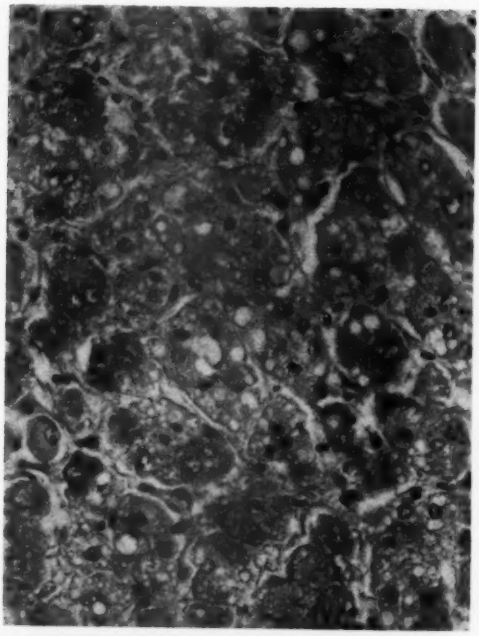

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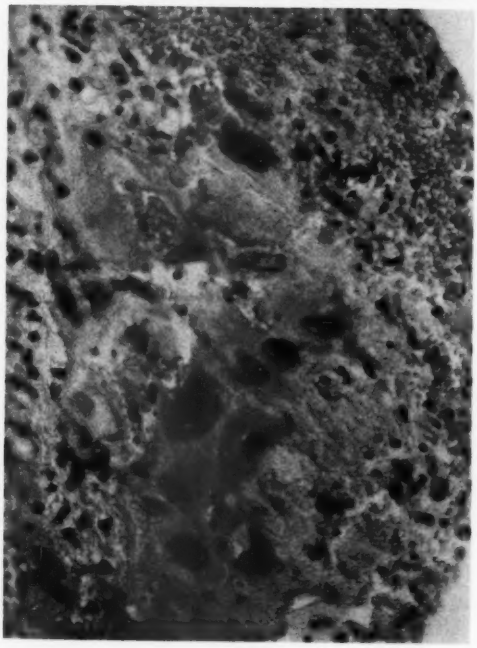

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FIG. 13. Ectromelia. Myocardium with limited infiltration by lymphocytes. Degeneration of the fibers. Fetus of a white mouse. × 190.

FIG. 14. Ectromelia. Myocardium with a slight infiltration by lymphocytes and granulocytes, with associated degeneration of the fibers of the muscles. Fetus of a white mouse. × 250.

FIG. 15. Ectromelia. Myocardium with infiltration by lymphocytes along a coronary vessel. Fetus of a white mouse. × 450.

FIG. 16. Ectromelia. Splenic necrosis with nuclear débris. Fetus of a white mouse. × 175.

FIG. 17. Rabies. Brain of chicken embryo with degeneration of nerve cells. × 350.

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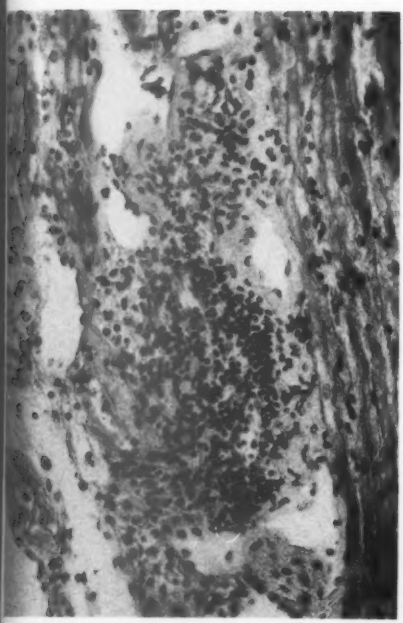

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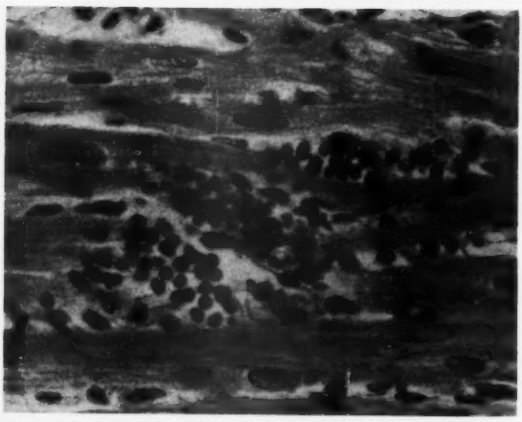

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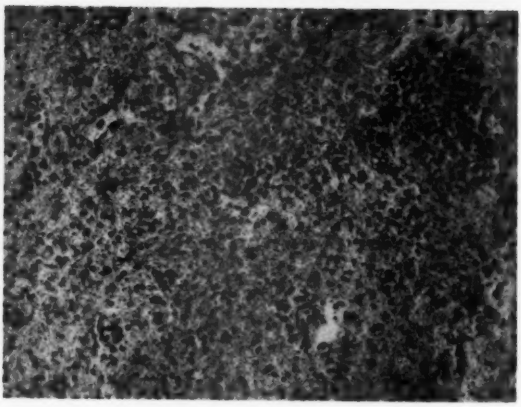

PHASE MICROSCOPE STUDIES OF RENAL GLOMERULI
GLOMERULAR DEPOSITS OF "HYALINE" SUBSTANCES *

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Phase microscopy has been used successfully in the study of unstained cells in smears, in teased fresh tissue (Richards,[1] Zollinger,[2]), and in frozen sections of fixed tissue (Oliver[3]). Recently Crossmon[4] and Bennett, Jupnik, Osterberg, and Richards[5] have shown that dehydrated and cleared paraffin embedded material, mounted in proper media, also can be advantageously examined under the phase microscope. In such preparations not only cellular details, but also thin fibers (reticulum, collagen, elastica) and cross sections of thin membranes (sarcolemma, basement membranes) become clearly visible.

Investigation of renal glomerular structure has been based largely upon procedures which demonstrate the capillary basement membrane and the reticulum fibers. These procedures vary from aniline blue staining and its modifications (McGregor,[6] v. Möllendorff[7]), to silver impregnations (Volterra,[8] Bensley and Bensley[9]), and periodic acid-Schiff's reagent technique (McManus[10]). They all have contributed to the present day concept of glomerular architecture. Because of its ability to bring out the capillary basement membrane and to show its relationship to the various normal and pathologic components, phase microscopy constitutes a valuable addition to this list. Other normal components of the glomerulus, the epithelial and the endothelial cells and the red blood cells, are clearly visualized. The localization of abnormal deposits and their inner structure, homogeneity, and refractility can be established readily. In the present study the phase microscope was applied to examination of glomerular deposits of homogeneous acidophilic substances, which are known under the generic name of hyalin, and which occur in a variety of diseases, such as amyloidosis, intercapillary glomerulosclerosis, arteriolosclerosis, and systemic lupus erythematosus.

MATERIAL AND METHODS

Our material consisted of kidney sections from 53 necropsies divided as follows: 16 cases of secondary amyloidosis, 18 of intercapillary glomerulosclerosis associated with diabetes, 11 of benign

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arteriolosclerosis, 5 of wire loop lesions in systemic lupus erythematosus, and 3 normal kidneys. Sections were cut at 5 μ and stained with hematoxylin and eosin, supplemented by aniline blue (Heidenhain's modification), and occasionally by periodic acid-Schiff's reagent, and crystal violet for amyloid. Kidneys from 5 cases of amyloidosis and 3 of each of the other groups, including the normal kidneys, were cut serially at 2 μ. Successive sections were stained as indicated, except that Gallego's modification* of aniline blue was substituted for Heidenhain's. One section of each series was used for phase microscopy.

The general technique and the principles of phase microscopy have been adequately dealt with in recent publications.[5] The phase microscope is used in essentially the same manner as the ordinary light microscope, except that it requires more careful attention to lighting and to alignment of optical elements. We had at our disposal a Spencer phase microscope equipped with "bright" and "dark" objectives of medium contrast. Illumination was provided by a ribbon filament, low voltage, microscope lamp. In general, we preferred a bright picture on a dark background. Since most of our work was done with media of high refractive index, we used "dark" phase objectives.

The selection of proper mounting media is of great importance. The refractive index of the medium should not differ too greatly from that

* Note by Editor. Gallego's procedure (*Trav. du lab. de recherches biol. de l'Univ. de Madrid*, 1919, 17, 95–110) differed from that currently in use by the authors, which was as follows:

1. Fix tissues in formalin or alcohol
2. Deparaffinize sections through distilled water
3. Stain and mordant for 5 to 10 minutes in:
   Distilled water, 50 cc.
   Carbol-fuchsin, 1 cc.
   Glacial acetic acid, 5 drops
4. Rinse in distilled water
5. Decolorize briefly in:
   Distilled water, 50 cc.
   Glacial acetic acid, 5 drops
   Formaldehyde (38 to 40%), 8 drops
6. Rinse in distilled water
7. Mordant for 35 to 45 seconds in 1% phosphomolybdic acid (longer mordanting will fade the nuclear stain)
8. Rinse well in distilled water
9. Stain for 1 to 2 minutes in:
   Distilled water, 50 cc.
   Aniline blue, 0.25 gm.
   Orange G, 1.0 gm.
10. Wash in distilled water
11. Dehydrate quickly through 95% and absolute alcohols, clear in xylol, and mount

Nuclei should be red; connective tissue fibers and basement membranes, blue; other tissue elements, yellow.

of the object, but should not be identical with it, lest the tissue become almost invisible. Media of higher index than that of the object will produce a picture of opposite contrast (bright or dark) to that with media of lower index. With dehydrated and cleared, paraffin-embedded tissues, the index should be either above or below 1.54. For this reason, the ordinary mounting media (Canada balsam, synthetic resins) are not suitable. Instead of solid mounts, we preferred fluids as suggested by Crossmon,[4] consisting of mixtures of diethylene glycol monobutyl ether (N = 1.44) with alpha-chloronaphthalene (N = 1.628), or alpha-bromonaphthalene (N = 1.656). With these mixtures, the refractive index could be varied at will between the two extremes. In general, we found the higher index media preferable, because of better definition of basement membranes and fibers. Before applying the mounting medium, sections were deparaffinized in xylol and then air-dried to remove the xylol. After each working period slides were returned to xylol. It is important that the sections be sufficiently thin, particularly when using media of high refractive index. Best results were obtained with sections cut at 2 μ.

Tissue Structure under the Phase Microscope

The appearance of various tissue elements under the phase microscope depends upon the thickness, refractive index, and width. Thickness and refractive index determine the optical path, which in turn determines the contrast between the object and the background. According to Wolter,[11] for optimal visibility the width of the object should bear a definite relation to the width of the slit of the phase plate. With a fixed slit, only objects of certain dimensions will be optimally reproduced. With the objectives used in this study, only narrow structures, such as nuclear membranes, basement membranes, reticulum, and thin collagen fibers were sharply defined. This also applied to striations of the voluntary muscles, perimysial membranes, and cytoplasmic granules. Wider structures were reproduced less perfectly, because of the appearance of halos along their edges, and loss of contrast toward the center. Pictures of wider objects should be interpreted with caution. For instance, a thickened basement membrane appears non-homogeneous, with highly refractile edges and substantially darker center. Tubular casts seem to be surrounded by a limiting membrane which is merely an optical effect. Some experience is necessary to be able to recognize these artefacts. Nevertheless, many fine details of the inner structure of larger objects can be visualized when stained preparations provide but little information.

THE NORMAL GLOMERULUS

The structure of the normal renal glomerulus has been discussed recently in great detail by several authors (McManus,[12] Allen[13]). We do not propose to give a complete description but to present the significant data obtained by phase microscopy, and discuss some of the controversial points. Under the phase microscope, the basement membrane of the capillary loops appears as a thin, sharply etched line, bright or dark, depending upon the contrast plate used (Fig. 1). The capsular basement membrane is similar in appearance, but thicker. In tangential sections there is appreciable loss of contrast because of increasing width; however, even then the membrane appears as an almost perfectly homogeneous band.

The capillary basement membrane surrounds the loop almost completely, except at the point of reflection onto the neighboring loops. Thus a small part of the circumference directed toward the center of the lobule may not be covered by the basement membrane. On occasion, it is possible to demonstrate gray, finely vacuolated or granular material in the center of the lobule. In the small segment devoid of basement membrane we could occasionally see an extremely thin line immediately beneath the endothelium, presumably representing the endothelial basement membrane.

The nature and the relationship of various glomerular components is not completely understood despite many detailed studies.[6-9,12,14,15] The capillary basement membrane is considered by some authors to be of endothelial origin,[13] though the majority believe it to be related to the glomerular epithelium.[6,9,12] It is continuous with the basement membrane of Bowman's capsule and follows the epithelium, reflecting from one loop to another. It is our impression that in addition to this epithelial basement membrane, there exists a separate endothelial membrane lying immediately beneath the endothelium. Ordinarily this membrane cannot be separated from the epithelial basement membrane, except where the latter is absent, *e.g.*, near the center of the lobule. However, under pathologic conditions the two membranes can be readily distinguished. Jones[16] expressed similar views based upon study of thin sections of renal glomeruli stained with periodic acid-leukofuchsin.

Another disputed point is the existence of intercapillary spaces, and of so-called mesangium.[14] According to one opinion,[13,17] the capillaries are completely surrounded by epithelium and basement membranes and lie free in the capsular space, attached only to the afferent and efferent arterioles. This is comparable to the position of the umbilical

cord in the amniotic sac. Obviously no intercapillary space can exist in this situation, because it would be identical with Bowman's space. A different view is advocated by those who believe that the capillaries are merely invaginated into Bowman's space and are incompletely covered by the epithelium and its basement membrane.[7,12,14] This situation would be analogous to the relation of the small intestine and its mesentery to the peritoneal cavity. Between the reflected layers of the basement membrane is the so-called intercapillary space. This space extends from the hilus into the most distant lobules. In the human kidney, it contains a small amount of connective tissue, mostly at the hilus, where it connects with the adventitia of the vessels. From the hilus, fine fibers and perhaps also fibroblasts fan out toward the periphery like a branching tree, gradually tapering off.[7,9] However, even the most peripheral parts of the intercapillary space must contain a minimal amount of some filling material, probably in the nature of ground substance of connective tissue. It seems probable that the gray vacuolated material visualized by the phase microscope, and the substance demonstrated by McManus, Lupton, and Graham[13] by means of special stains represent the contents of the intercapillary space.

"HYALINE" DEPOSITS IN GLOMERULI

Amyloid Deposits. Under the phase microscope deposits of amyloid appear as non-homogeneous, gray, spongy masses, filled with numerous small, scattered vacuoles. The vacuoles are strikingly brought out by the phase method, though on close scrutiny they can be discerned even in stained preparations. This vacuolated appearance is in itself not specific for amyloid, because other substances such as the hyalin of intercapillary glomerulosclerosis may have similar structure, particularly in early stages. As in stained preparations, larger deposits of amyloid show a tendency to fragmentation and rippling on sectioning. For definite identification specific stains are required.

In most of our cases, main deposits of amyloid are seen near the hilus, surrounding the infundibulum (Figs. 5 and 6). From this region, finger-like projections extend toward the periphery of the glomerulus between the capillary loops. In later stages these projections become thicker and longer, while the loops are straightened and stretched around the masses of amyloid (Figs. 8, 9, and 10). Eventually, the lumina of the loops become compressed or entirely obliterated. In this process, the amyloid may infiltrate into the basement membrane which loses its distinctive appearance and can no longer be recognized as a separate structure. In addition to the hilar amyloid,

one can often observe small deposits about the basement membranes of the capillary loops, apparently not connected with the hilus. In two of our cases, most of the amyloid was found in the peripheral loops rather than near the hilus, lying between the epithelial and the endothelial basement membranes and narrowing the capillary lumina.

The exact localization of amyloid deposits in the glomeruli has been a matter of discussion. Bell[19] believed that amyloid first appears on the inside of the basement membrane and gradually fills the lumen of the capillaries. According to Fahr[20] and Allen,[13] the deposits form nodular or band-like swellings in the capillary walls, which are caused by "local conversion of pre-existing reticulin or collagen."[13] On the other hand, in his discussion of primary senile degeneration of the glomeruli, Kimmelstiel[21] mentioned that in amyloid degeneration there occurs thickening of the intercapillary connective tissue axis. From our findings it seems likely that amyloid which is deposited around the infundibulum lies within the connective tissue core, that is, the mesangium of the glomerulus. From this location it extends directly between the loops, following the intercapillary spaces. The distribution of amyloid closely resembles that of reticulum fibers in the glomerulus as illustrated by Bensley and Bensley,[9] in their Plates 4 and 5.

Intercapillary Glomerulosclerosis. Under the phase microscope, the typical Kimmelstiel-Wilson lesions appear as nodules of vacuolated or lamellated material lying in the centers of glomerular lobules and surrounded by capillary loops. These nodules never occur on the side of the capillary facing Bowman's space, but only on the "inner" surface of the loop. They are separated from capillary lumina by thin basement membranes (Figs. 11 and 12). In more advanced lesions the structure is distinctly lamellated, while the surrounding capillaries are compressed and obliterated (Figs. 13 and 14). All stages of transition can be found between the spongy and the lamellated nodules.

In addition to the typical lesions, the same spongy vacuolated material is found in the form of elongated deposits lying between the capillary loops and often extending to the hilus. Bell[17] was the first to point out the existence of this diffuse form of intercapillary glomerulosclerosis, occurring independently or in combination with the peripheral nodules. These findings are supported by the studies of McManus.[12] It is our impression that nodular lesions are always associated with diffuse deposits and represent the more advanced form of the latter.

The problem of the exact location of the Kimmelstiel-Wilson lesions was thoroughly reviewed by Allen,[22] who favored intramural rather than intercapillary origin. The same view is shared by Bell.[17] On the other

hand, the studies of McManus[12] as well as our own findings outlined in the preceding paragraphs support the original contention of Kimmelstiel and Wilson[23] that the deposits truly are localized in the intercapillary space.

Hyalin of Arteriolosclerosis. In severe arteriolosclerosis, particularly in diabetic patients, afferent arterioles of glomeruli may show conspicuous deposits of homogeneous, strongly eosinophilic material (Figs. 15 and 16). Similar material often may be seen in the glomerular loops (Figs. 13 and 14), though this fact is seldom mentioned in the standard textbooks (Fahr[20]). Under the phase microscope, the material is highly refractile and homogeneous, except for occasional small vacuoles. It is our impression that it first appears under the capillary endothelium, that is, between the epithelial and the endothelial basement membranes. The latter is then rapidly destroyed and the markedly dilated loop becomes filled with hyalin. The epithelial basement membrane of the involved capillary is not affected and can be demonstrated even in advanced lesions (Figs. 13 and 14).

Bell[17] emphasized that severe hyalinization of arterioles is strong presumptive evidence of diabetes mellitus. This is particularly true when efferent arterioles are involved in addition to the afferent ones.[22] Apparently similar significance, at least on the basis of our material, can be ascribed to extensive intraglomerular deposits of hyalin. As might be expected, glomeruli bearing intracapillary hyalin often contain also lesions of intercapillary glomerulosclerosis. Some kidneys show heavy hyaline deposits with very little intercapillary sclerosis, while the reverse is true of many other cases. When both lesions occur in the same glomerulus, they can be distinguished readily, particularly by their appearance under the phase microscope (Figs. 13 and 14).

Wire Loop Lesions in Systemic Lupus Erythematosus. Wire loop lesions are considered to be characteristic alteration of kidneys in lupus erythematosus. Their staining reactions have been studied by many authors.[12,13,24] With hematoxylin and eosin, they are strongly acidophilic and give the impression of thickened basement membranes (Fig. 3). After periodic acid-Schiff's reagent, they stain red, though perhaps less intensely than the basement membrane proper. On the other hand, the Mallory-Gallego stain colors the basement membrane dark blue, while wire loops vary from grayish blue to yellow. With this technique, one can demonstrate that the basement membrane usually remains unchanged and that the wire loop is located between the epithelial basement membrane and the capillary endothelium. This point can also be brought out well with the phase microscope, which shows typical wire loops as homogeneous, refractile bands with only a

few scattered vacuoles (Figs. 2 and 4). Some bands may be broken into several irregular segments. Earlier lesions are less homogeneous and show large vacuoles. The material of wire loops is slightly more refractile than hyalin of arteriolosclerosis, but distinctly less refractile than the basement membrane proper. The latter appears as a thin even line on the outside of the wire loop. Occasionally, the basement membrane cannot be differentiated from the wire loop material, and appears actually infiltrated by the latter. This is similar to the process observed in amyloidosis. Endothelial nuclei frequently can be seen on the inside of the wire loops. At times, a thin black line, representing the endothelial basement membrane, appears between these nuclei and the wire loops (Fig. 4).

Within the lumen of the capillaries one can find deposits of refractile homogeneous material, similar to that of wire loops. With the Mallory-Gallego stain, this material is bright yellow. It often contains fragments of hematoxylin-staining substance ("hematoxylin bodies"). These deposits are usually designated as thrombi, though they have been interpreted also as parts of wire loops protruding into the lumen.[13] They may be attached to wire loops, but they also may appear independent of the latter.

The nature of the wire loop lesions has been variously conceived. Klemperer, Pollack, and Baehr[24] originally believed them to be a form of "fibrinoid" alteration of the capillary wall with formation of tryptically digestible material between the endothelium and the epithelial basement membrane. Bell[17] considered them a simple patchy thickening of the basement membrane, while McManus[12] stated that wire loops are deposits of hyaline material under the endothelium. Similarly, our findings suggest that wire loop material is deposited between the epithelial and the endothelial basement membranes. Recently Klemperer *et al.*[25,26] put forward the idea that this material may be a product of degradation of nucleoproteins.

Summary and Conclusions

Phase microscopy lends itself readily to the study of renal glomerular architecture in fixed, paraffin-embedded material. With the aid of mounting media of high refractive index, this technique allows simultaneous visualization of various structures and demonstrates particularly well the fibrillar and membranous constituents, such as reticulum fibers and capillary basement membranes. By combining phase microscopy with various special stains applied to serial sections, fine details of normal histology, as well as location and inner structure of abnormal deposits can be shown. This procedure was applied to the study of

normal glomeruli and of hyaline deposits in amyloidosis, intercapillary glomerulosclerosis, arteriolosclerosis, and systemic lupus erythematosus.

The hyaline deposits differ in their inner structure, as well as in their location in relation to the walls of the capillary loops.

In systemic lupus erythematosus the deposits, so-called wire loops, lie in the capillary wall between the endothelial and the epithelial basement membranes. They are homogeneous and highly refractile.

In arteriolosclerosis, the hyaline deposits first appear under the endothelium and later fill the lumen. They are also homogeneous in structure and only rarely show small vacuoles.

Amyloid deposits are found occasionally in the capillary wall, in the same location as wire loops, and more often between the capillaries, presumably in the intercapillary space. These deposits are non-homogeneous, spongy, or finely vacuolated.

In intercapillary glomerulosclerosis, finely vacuolated material appears between the capillary loops. Typical Kimmelstiel-Wilson nodules lie in the centers of glomerular lobules and have a vacuolated or lamellated structure.

The distribution of these hyalins and their relation to the capillary wall and to the vascular root of the glomerulus are in accordance with the concept of the intercapillary space, as advocated by Zimmermann, v. Möllendorff, Bensley, and McManus.

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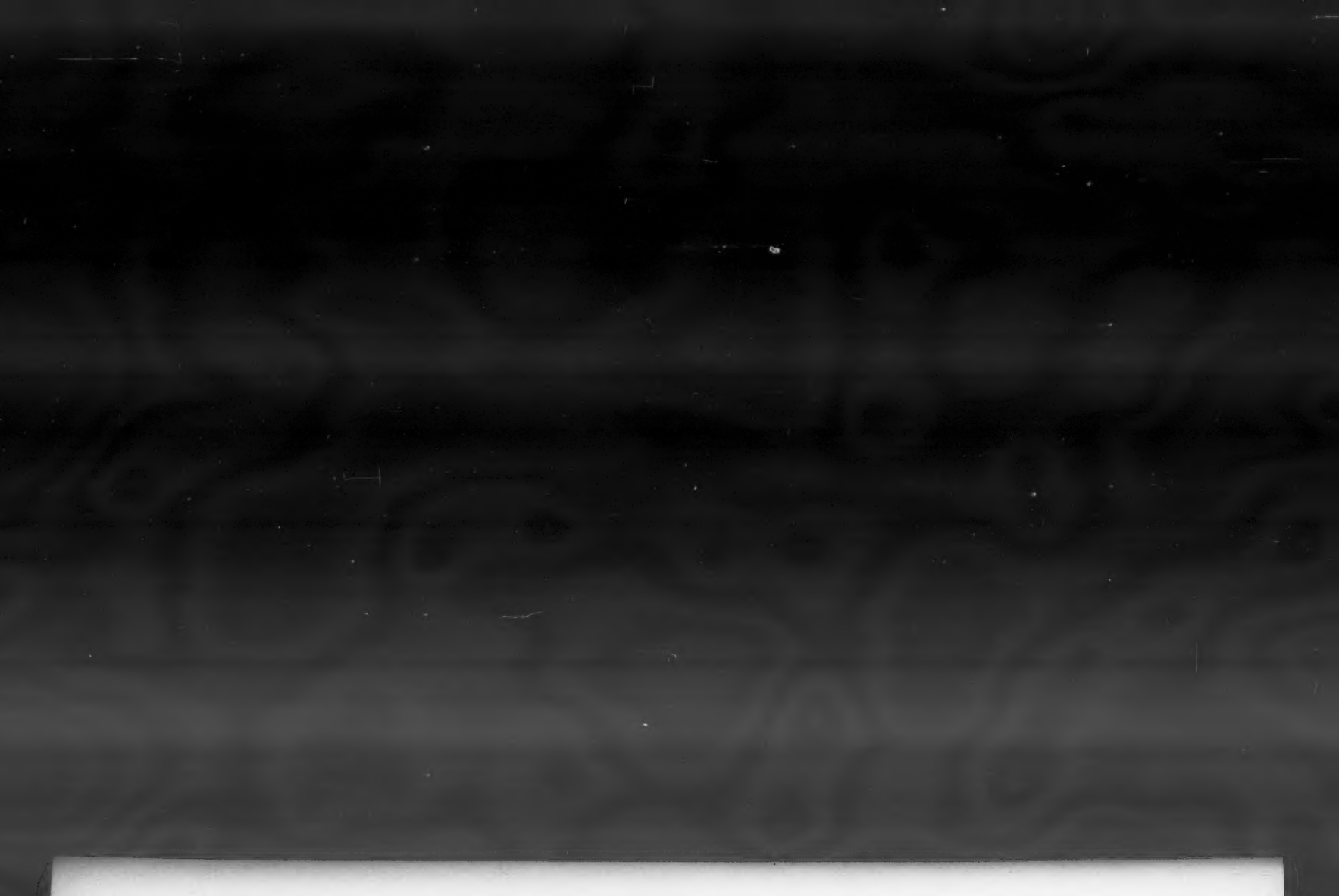
LEGENDS FOR FIGURES

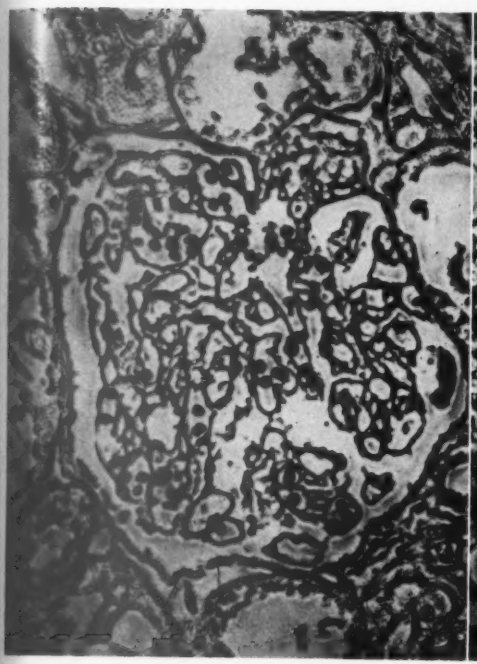
FIG. 1. Normal human glomerulus under phase microscope. Basement membrane outlined in black. Black dots in capillary lumina represent red blood cells. × 325.

FIG. 2. Glomerulus in lupus erythematosus disseminatus under phase microscope. Two wire loops may be noted, one near the center, the other in the eleven o'clock position. × 325.

FIG. 3. Wire loops in lupus erythematosus at a high magnification. Hematoxylin and eosin stain. × 860.

FIG. 4. Same wire loops as in Figure 3. Phase microscopy. Dark gray material of capillary walls is indicated by arrows. × 860.





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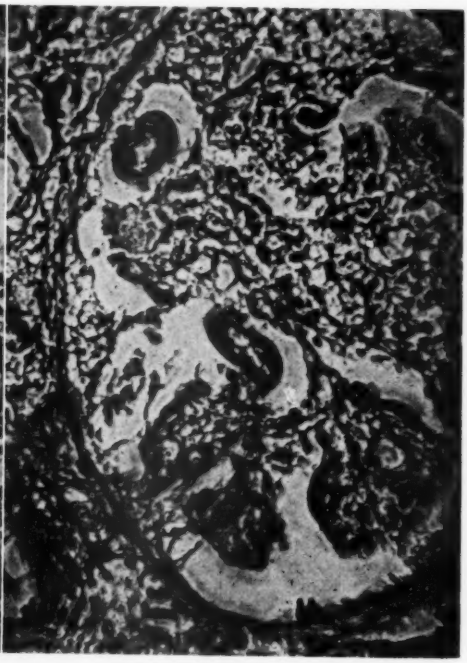

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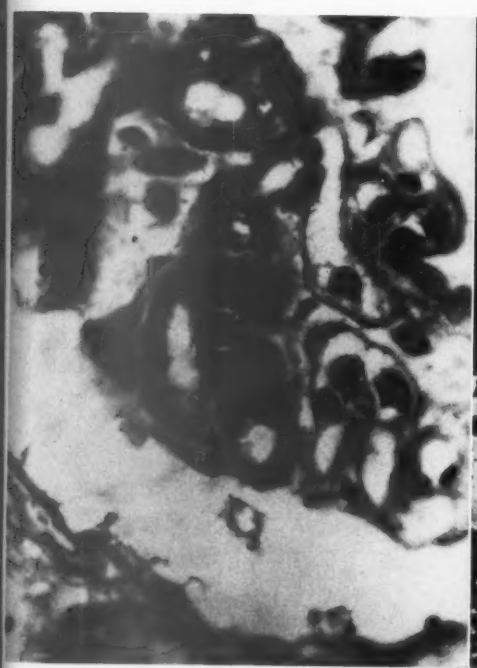

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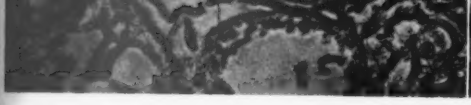

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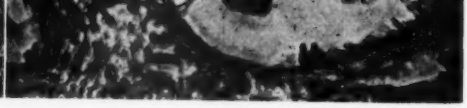

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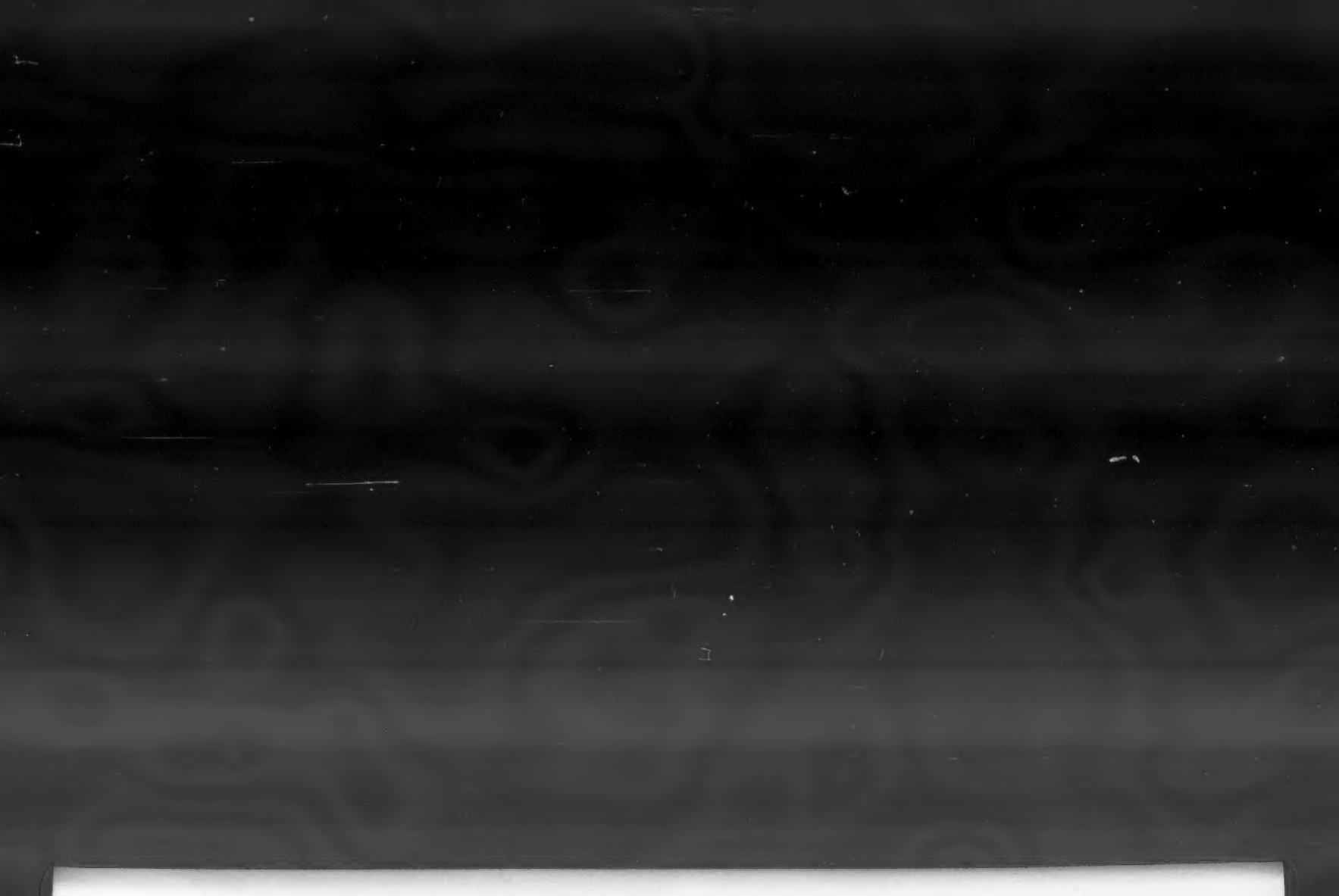
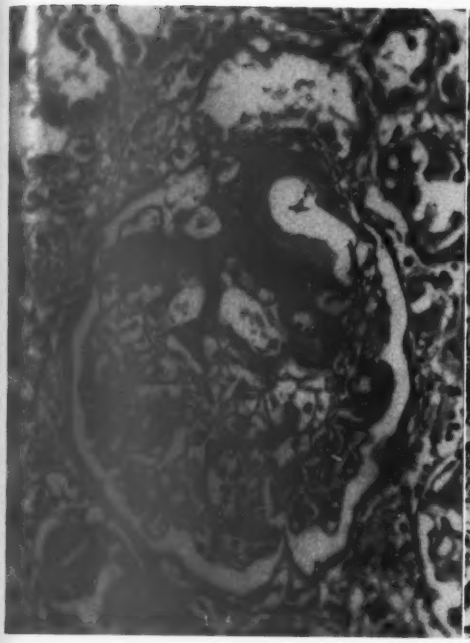
FIG. 5. Glomerulus in renal amyloidosis. Amyloid deposit at the hilus. Periodic acid-Schiff's reagent. × 240.

FIG. 6. Same glomerulus as in Figure 5. Phase microscopy. × 240.

FIG. 7. Same as Figure 6 at a higher magnification. Of note is the spongy appearance of the amyloid (arrows). Phase microscopy. × 835.

FIG. 8. Glomerulus in advanced amyloidosis. Periodic acid-Schiff's reagent. × 240.





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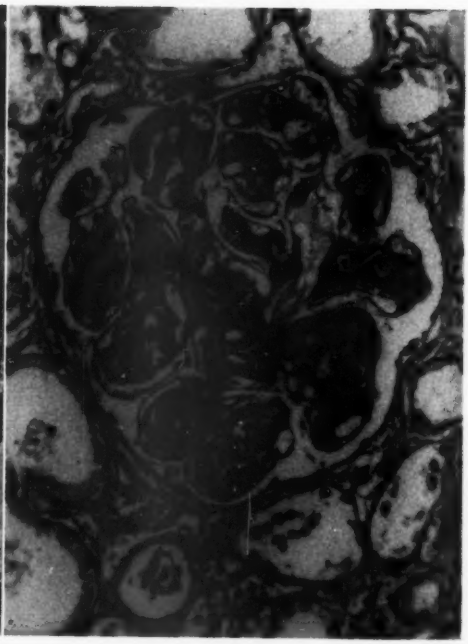

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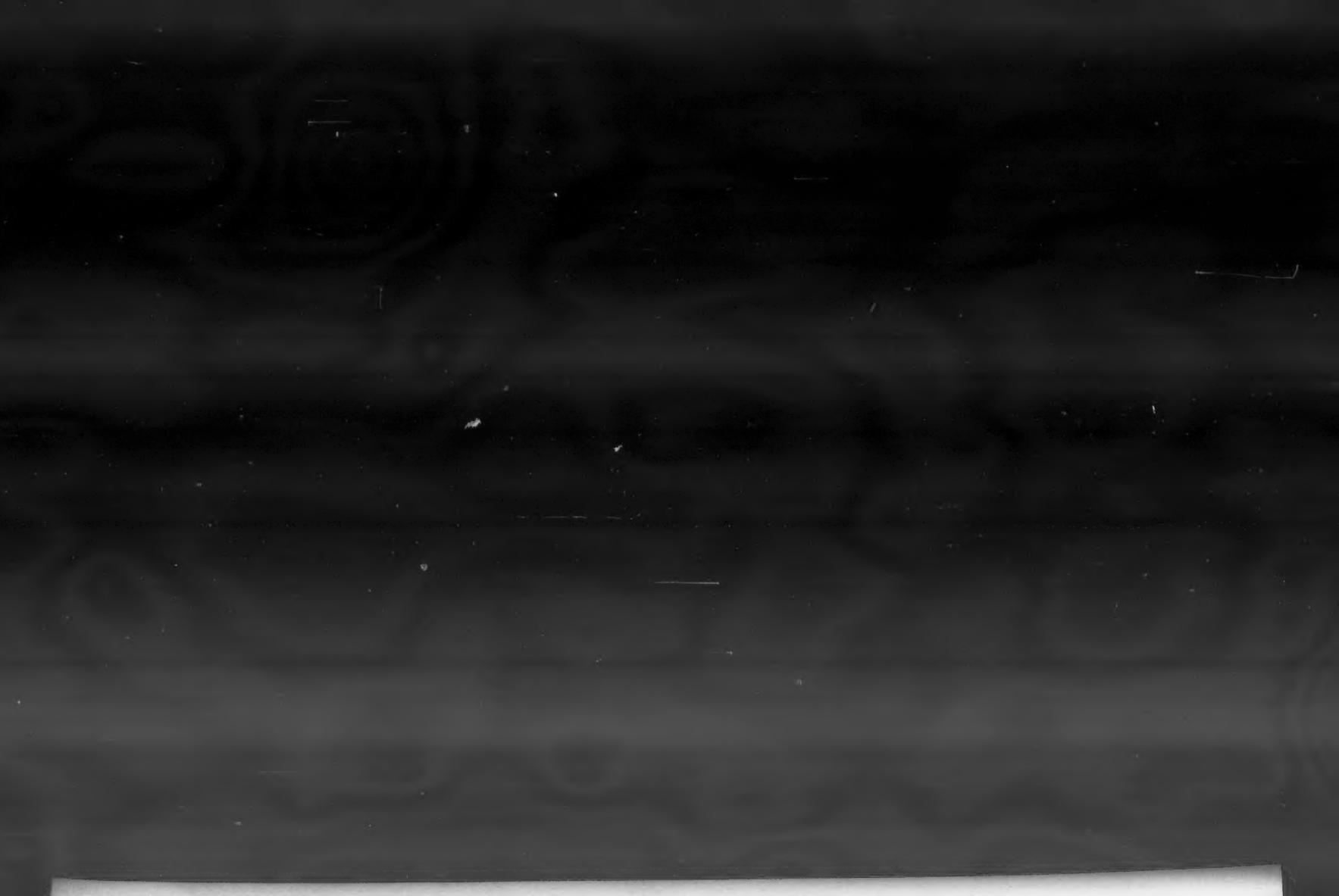
FIG. 9. Same glomerulus as in Figure 8 at a higher magnification. Amyloid occupies the center of the lobule. Periodic acid-Schiff's reagent. × 870.

FIG. 10. Same as Figure 9. Phase microscopy. × 870.

FIG. 11. Intercapillary glomerulosclerosis. "Hyaline" nodule surrounded by a wreath of capillaries. × 870.

FIG. 12. Same as Figure 11, showing spongy structure of the nodule. Of note is the thick outer and thin inner basement membrane of capillaries. Phase microscopy. × 870.



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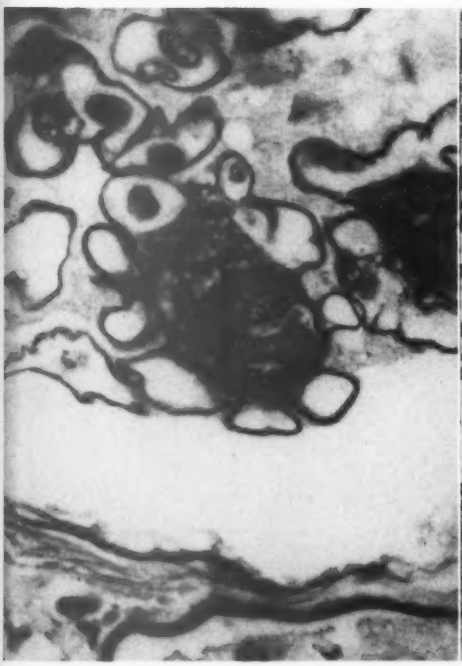

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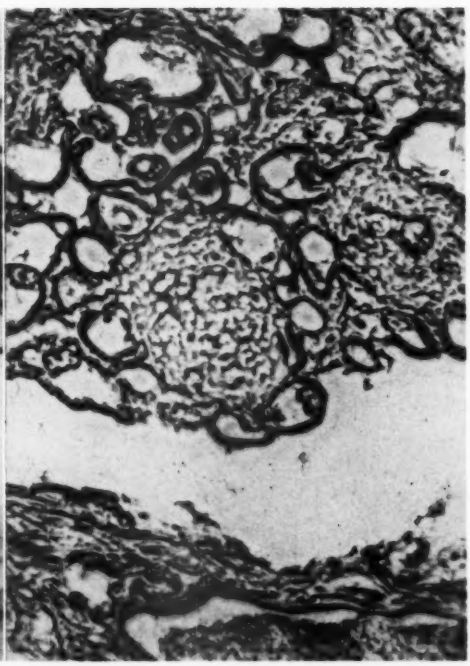

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FIG. 13. Part of a glomerulus in diabetes mellitus. There is a lamellated nodule of intercapillary glomerulosclerosis in the center and a capillary loop filled with homogeneous hyalin in the upper part of the field. Periodic acid-Schiff's reagent. × 385.

FIG. 14. Same as Figure 13. Note parallel "knife marks" in the upper hyaline nodule. Phase microscopy. × 385.

FIG. 15. Afferent arteriole showing hyaline mass within the wall. Periodic acid-Schiff's reagent. × 385.

FIG. 16. Same as Figure 15. There is a similarity to the hyaline substance seen in Figure 14. Phase microscopy. × 385.

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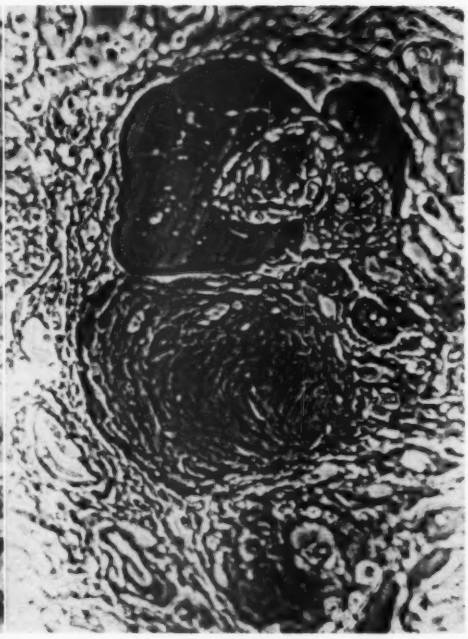

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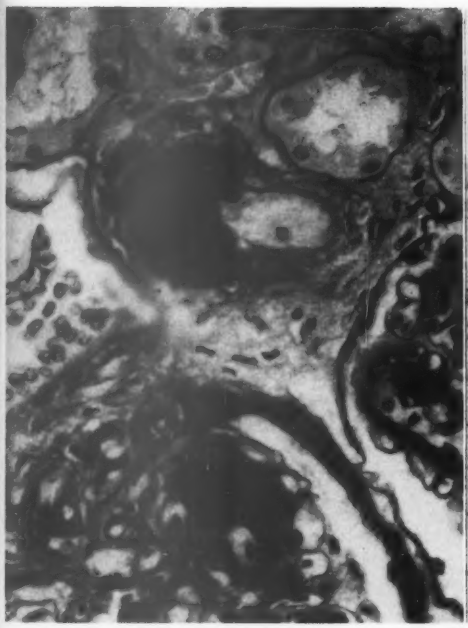

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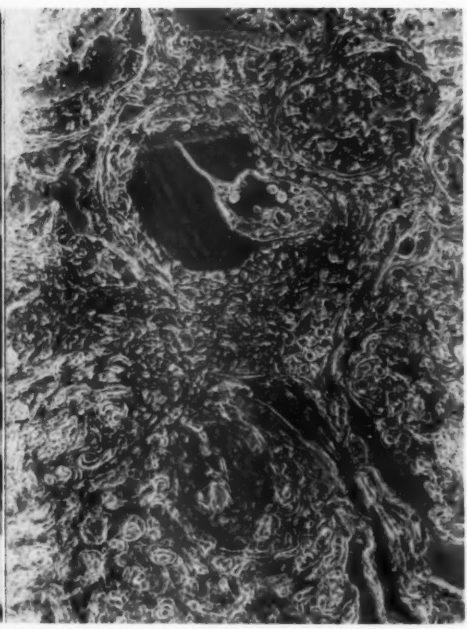

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FACTORS FAVORING PHAGOCYTOSIS BY RETICULO-ENDOTHELIAL CELLS EARLY IN INFLAMMATION *

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The term phagocytosis will be used to comprise the approach and contact between particulate substances and phagocytes, as well as the final passage of the substances into the bodies of the cells. It will not be used to include the subsequent effects upon either phagocytes or particles. Comprehensive reviews from various aspects have been presented by Mudd, McCutcheon, and Lucké[1] in 1934 and by Berry and Spies[2] in 1949. Other contributions on the subject will be noted only when pertinent.

The factors concerned in phagocytosis, or entrance of particulate substances into cells, may be divided into those which bring the phagocytes and particles into contact and those which influence entrance of particles into phagocytes once the two have been brought into contact.[3] Those which influence contact between particles and phagocytes may be listed as follows: chemotaxis, temperature, any means which acts mechanically to bring cells and particles together, the surface charges of cells and particles,[4] humoral factors which serve to modify the surfaces of particulate substances[2] and, finally, such physical characteristics of the surfaces of phagocytes as stickiness and roughness. The factors which influence entrance of particulate substances into cells, once the two have been brought into contact, include size of particles, ameboid movements of the phagocytes, the characteristics of the surfaces of both particles and phagocytes, and any forces which serve to rub, or press, the substances against the phagocytes. The last method has been termed surface phagocytosis by Wood, Smith, and Watson[5,6] and has been beautifully demonstrated in the case of polymorphonuclear cells rubbing against each other, and against the walls of the pulmonary alveoli. That "surface phagocytosis" functions also in the case of the mononuclear phagocytes will be demonstrated in the present contribution. In addition to these four general methods by which particulate matter attains entrance into phagocytes, two other methods have been demonstrated in the case of lipids,[3] at least. These are: (1) absorption, or solution, of an otherwise impenetrable lipid into a natively penetrable lipid[7]; and (2) con-

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version of an impenetrable lipid into a penetrable compound by enzymatic action at the surfaces of cells.[8]

Phagocytosis on the part of the large mononuclear cells of subcutaneous tissues within the first 12 hours of inflammation will be considered from these various angles. Normally subcutaneous tissue contains only fibrocytes (fibroblasts), mast cells, common endothelium, and resting-wandering cells.[8,9] The last represent somewhat fixed forms belonging to the reticulo-endothelial system,[9] and are the only cells of the group which are endowed with capacity for phagocytosis. Hematogenous invaders are rare in normal subcutaneous tissues. Polymorphonuclear leukocytes enter the tissues in great numbers within 2 to 3 hours of irritation, and small mononuclear cells within 9 hours.[8,10,11] The latter develop high degrees of phagocytic activity within about 6 hours thereafter. It is obvious, therefore, that only the resting reticulo-endothelial cells are available for phagocytosis of invading particles, whether through entry of harmful particles from without or by parenteral administration of therapeutic substances, during the period preceding the arrival of polymorphonuclear cells, and only they are available during the first 12 hours of inflammation for phagocytosis of the many substances not phagocytosable by polymorphonuclear cells. It is remarkable that investigation of the factors influencing phagocytosis by these cells during the early hours of inflammation should be so scanty, considering their importance in the early defense of the organism against invaders.

Experimental Procedures

The experiments were carried out both *in vivo* and *in vitro*. They were performed on adult, male mice, C3H strain, about 6 months of age. The material used to demonstrate phagocytosis were: India ink; a birefringent lipid known as protagon[12]; washed, homologous erythrocytes; and autoclaved, washed, human tubercle bacilli.* These were suspended in 5 per cent glucose, or in mixtures of glucose or Locke's solutions with homologous serum. The diluents, and the proportions of suspensoid for any given set of experiments, are presented in Table I.

The *in vivo* experiments were carried out by subcutaneous injection of 0.5 cc. of the experimental suspension into two areas of the same mouse at 1 to 2½ hours, and 4 to 5 hours, respectively, before sacrifice. The former were the control areas. The injections were given into

* We are grateful to Dr. Harold Kenton and his staff, Department of Bacteriology, New England Deaconess Hospital, Boston, Mass., for the supply of bacteria used for these experiments.

the lower dorsal and abdominal regions, interchangeably. Pentobarbital sodium was given intraperitoneally at the time for sacrifice, and the mice were exsanguinated by cardiac puncture. The serum was saved for use in succeeding experiments. Bits of the connective tissue were excised from each area immediately following death, spread as films on coverglasses, and either fixed or moistened with serum containing neutral red for supravital study.[13] The films from the experiments with lipid were fixed in formaldehyde gas, mounted in phenol

TABLE I

Proportions of Suspensoids in Diluents

	In vivo experiments		*In vitro* experiments	
Suspensoid	Diluent	Average proportion of suspensoid to 1 cc. of diluent	Diluent	Average proportion of suspensoid to 1 cc. of diluent
Lipid (protagon)	5% glucose	1-3 mg.	Serum-glucose (5%) (4:1)	1 mg.
India ink	5% glucose	0.04-0.07 cc.	Serum	0.3-0.5 cc.
Tubercle bacilli	5% glucose	5,520,000	Serum, or serum-Locke's solution (1:1) or (1:3)	5,520,000
Homologous red blood cells	Homologous serum, or Locke's solution, or 5% glucose	250,000–600,000	Serum-Locke's solution (1:1)	250,000

gelatin, and examined with polarized light. The others were fixed in Carnoy-Lebrun solution for 2 hours and then stored in 80 per cent alcohol. The films from experiments using tubercle bacilli were stained by the Ziehl-Neelsen method for acid-fast bacteria. The remainder were stained with hematoxylin and eosin.

The *in vitro* experiments were carried out on films from subcutaneous areas, which had been injected with 5 per cent glucose 4 to 5 hours before sacrifice in order to assure the presence of neutrophils. Technical difficulties made it inadvisable to inject the control areas, and films from normal, uninjected areas were consequently used as controls. Sacrifice was carried out as described in the *in vivo* experiments. All films were covered with the experimental suspension as soon as they were made and were placed, suspension side up, in moist Petri dishes. All were incubated 1½ hours at 37° C. Some were then inverted upon slides, stained with neutral red for supravital study, and examined without further manipulation. The remainder were inverted onto slides and fixed in 10 per cent formalin (prepared with physiologic saline solution) overnight, followed by 1 hour in 80 per cent alcohol and then 1 hour in Carnoy-Lebrun solution, from which they were

stored in 80 per cent alcohol. These were stained as described for the *in vivo* experiments.

All films were examined microscopically and estimated from three standpoints: first, qualitative characteristics of the resting-wandering cells, including contraction, motility, presence of "fluid vacuoles,"[13] presence of fat, characteristics of the phagocytic vacuoles, and evidences of normalcy or of injury; second, degree of infiltration of polymorphonuclear leukocytes; and third, degree of phagocytosis of the experimental suspensoid on the part of both polymorphonuclear and resting reticulo-endothelial cells. The supravital films afforded a better understanding of the qualitative characteristics of the cells. The other information could be obtained equally well by either method of study. The fixed films allowed preservation. The photographs are from them only. The number of definitive experiments performed with each suspensoid, and the grade of phagocytosis toward each on the part of the local reticulo-endothelial cells are summarized in Table II. Actually,

TABLE II

Estimated Grades of Phagocytosis by Resting-Wandering Cells *

Suspensoid	*In vivo* experiments			*In vitro* experiments		
	Number of experiments	Average grade of phagocytosis		Number of experiments	Average grade of phagocytosis	
		Experimental area	Control area		Experimental area	Control area
Lipid (protagon)	2	++++	+	3	+++	±
India ink	4	++++	++	3	++	+
Tubercle bacilli	1	+++	+	4	+	±
Homologous erythrocytes	5	++	±	3	±	o

* See the last paragraph of the section on Experimental Procedures for a full explanation of the figures used in this table.

more experiments were performed in each series of studies than is indicated by Table II. They represented efforts to determine the concentration of any given suspensoid that was best suited for demonstration of phagocytosis, choice of the best dye or bacterium to elicit phagocytosis under the specific experimental conditions, and, in particular, attempts to modify intact erythrocytes so as to make them phagocytosable under *in vitro* conditions without impairment to their demonstration in sections. Once these experimental variables were controlled, demonstration of phagocytosis became certain and repeatable in all cases, except in the *in vitro* phagocytosis of erythrocytes.

That remained a matter of unpredictable success. It seems more exact, therefore, to tabulate merely the number of successful experiments, with any given suspensoid, once optimum conditions were determined, than to give a false concept of multiplicity by including all experiments that were carried out in each series.

Experimental Findings and Discussion

The films from all of the experimental areas, regardless of type of experiment or of suspension, were characterized by prolific infiltrations of polymorphonuclear cells (odd numbered illustrations). This was expected, and was so planned, since sacrifice occurred uniformly 4 to 5 hours after injection of either the glucose for the *in vitro* experiments, or of the suspension for the *in vivo* experiments. The leukocytes were young, actively ameboid, and showed no indications of degeneration at that time. They demonstrated little phagocytosis of the various suspensoids, except of the bacilli, despite the vigorousness with which they moved over and among the particles thereof. This is in agreement with the observations of Clark, Clark, and Rex[14] following injection of some of the same substances into intact, living animals, and of Tompkins[8] in mice following injections of cholesterol. What phagocytic activity they demonstrated was always intensified by prolongation of exposure to the particular suspensoid.

The films from the experimental areas, both *in vivo* and *in vitro*, and also those from the control areas *in vivo*, were likewise characterized by specific modifications of the resting reticulo-endothelial cells, which can be looked upon as mobilization, or activation, in preparation for phagocytosis. The cells had contracted from their normal expanded and rather fixed status to become free; they had shortened and thickened in the process, even to rounding, and the ectoplasm had become contracted into dense peripheral rims. They usually still contained the "fluid vacuoles" characteristic of their normal status,[13] but these were smaller than normal and the cytoplasm of the cells themselves had lost the optical clarity typical of the normal and had become more opaque and more refractive than normal. Most of these modifications are characteristic of the conversion of resting-wandering cells into macrophages under the influence of ions of potassium free from the inhibitory factors of serum.[13] Whether cytologic injury was sufficient to allow escape of potassium at the early hours of these experiments is unknown. It has been shown to be present at later stages of inflammation.[15] If it is free in the first hours of inflammation, the modifications of the resting-wandering cells in these experiments are easily

explainable.[13] The experimental procedures were such as to have exposed the films to any liberated potassium considerably ahead of exposure to serum.

These modifications of the resting reticulo-endothelial cells are such as would seem obviously to promote phagocytosis. Fenn[16] has demonstrated that a necessary prerequisite to phagocytosis consists of decrease in the surface tension of phagocytes, or of increase in that of particles to be phagocytosed. Contraction of expanded bodies to more rounded forms is customarily attended by decrease in their surface tension, and it would seem likely that this occurs in the case of these contracted resting-wandering cells. The concomitant denseness of the cytoplasm, and the bogginess and irregularities of their ectoplasm would seem to offer increased adhesive qualities toward particulate substances brought into contact with them by any mechanism. Finally, if the content of their "fluid vacuoles" consists of water and proteins, as seems likely, excretion of the same should serve to develop cytoplasmic edema within the cells, and to add spreading factors to the humoral environments of the suspensoids without the cells.[13] These would, in turn, serve to decrease the surface tension of the suspensoids.

Although free and rounded, and exhibiting the characteristics that have been discussed as favorable to adhesion, these mobilized phagocytes presented no evidence of motility. They must not, therefore, have been chemotactic at that period, and it becomes necessary to turn to other phases of the experiments to understand how the degree of contact was achieved which was obviously necessary for the abundant phagocytosis which they displayed (Table II). The lack of chemotactic activity on the part of the large mononuclear cells in these experiments is in agreement with the findings of Mudd, McCutcheon, and Lucké[1] *in vitro*. It is, however, contrary to the findings of Clark and Clark *in vivo*. The latter workers observed active chemotaxis by these cells in the tails of amphibia[11] and the ears of rabbits.[14,17] It seems possible that the brevity of the experimental periods, or the method of observation in the present studies and in those of Mudd *et al.*, were less favorable to this activity than the prolonged *in vivo* conditions of the experiments by Clark and Clark. Regardless of the explanation, however, it is obvious that phagocytosis on the part of the activated resting-wandering cells in the present experiments was achieved by virtue of forces other than chemotaxis.

Phagocytosis by the resting reticulo-endothial cells was common to all experimental areas, and, except in the case of erythrocytes, to the control areas, in lesser degree. This is true whether or not the polymorphonuclear cells also phagocytosed the particular suspensoid, and

is in accord with the findings of Good and Good in rabbits.[18] The degree of phagocytosis under any specific set of experimental conditions, however, varied with the time of exposure of the tissues to the suspension and with the particular suspensoid. This is in accord with long accepted conclusions on these points.[19-21] The resting-wandering cells, whether activated or as normal control cells, phagocytosed more of a given suspensoid under any set of conditions the longer the time of exposure. This was equally true of the polymorphonuclear cells in those cases in which they did phagocytose. Time alone, of course, can account only for variations in amount of phagocytosis. It cannot account for the attainment of cohesion between suspensoid and cells which is necessary for phagocytosis, nor for the other factors involved in phagocytosis. Once the resting-wandering cells had been activated in these experiments, time allowed merely a longer exercise of the factors involved in bringing the suspensoids into contact with them. It will be shown that these are factors concerned with "surface phagocytosis."

The long recognized relationship between particulate size and degree of phagocytosis also was in evidence in these studies. This may be seen from Table II. The conditions under which the *in vivo* experiments were conducted are quite comparable to those in many reports on vital staining or on phagocytosis.[21,22] The conditions under which the *in vitro* experiments were conducted are more unique. Both agree, however, in supporting the conclusion that the degree of phagocytosis under any set of experimental conditions is dependent upon particulate size. It may be seen from Table II that the degree of phagocytosis *in vivo* decreased from a maximum with the lipid (protagon), through India ink and tubercle bacilli to a minimum with homologous erythrocytes. While the degree of phagocytosis was less *in vitro* than *in vivo* with any given suspensoid (*cf.* Figs. 1 to 8 with Figs. 9 to 16), the same quantitative sequence holds between individual suspensoids, nonetheless. Exact measurements of particulate size are not available. The lipid formed a perfect emulsion in which individual particles were not visible under 900 × magnification in the light microscope, and which merely glowed as a diffuse whole under polarized light (Figs. 2 and 10). The individual particles of carbon in the emulsions of India ink usually aggregated somewhat to form larger particles, but the smallest were just visible at the above magnification (Figs. 4 and 12). The bacilli were obviously many times the size of these, and the erythrocytes, again, many times the size of the bacilli. Particular size, therefore, although affecting the degree of phagocytosis, as is true of duration of exposure, does not in itself explain the methods by which

particles and activated macrophages were brought into contact. Explanation for contacts in the early hours of inflammation before development of motility on the part of the activated resting-wandering cells, or of arrival of ameboid mononuclear phagocytes from the blood, must still be sought in other phases of the experiments.

Comparison of the degree of phagocytosis of any given suspensoid *in vitro* with that *in vivo* shows that it was uniformly greater *in vivo* (Figs. 1, 3, 5, and 7 *vs.* Figs. 9, 11, 13, and 15). Suspension, activation of the macrophages, duration of exposure, and temperature were comparable in both sets of experiments. The cells appeared as viable in the supravital preparations *in vitro* as *in vivo,* the polymorphonuclear cells moved as actively, the staining of the phagocytic vacuoles with neutral red was similar. These cells appeared to be as capable of phagocytosis in the *in vitro* as in the *in vivo* films. Yet they consistently phagocytosed more *in vivo* than when exposed for the same length of time *in vitro*. The explanation for this divergence seems to lie in the muscular activity that underlay the *in vivo* experiments in contrast to the lack of movement after exposure of the films to the suspensoid *in vitro*. It seems reasonable to suppose that the constant play of muscles beneath the injected areas, *in vivo,* led to more or less contact and rubbing between suspension and cells of the area. The mononuclear cells activated for phagocytosis would thus have been brought into far more frequent contact with the suspended particles *in vivo* than *in vitro* even though the cells in both areas were equally activated and exposed for equal periods of time. The cells *in vivo,* in other words, were subjected to "surface phagocytosis" by movements which were lacking *in vitro*. Muscular movement, then, would seem to be one of the forces aiding in phagocytosis by the resting reticulo-endothelial cells of connective tissues in the early hours of inflammation. The activation of the cells in the manner described would make them especially receptive to, and retentive of, particles thus rubbed against them by the play of underlying muscles. The significance of this finding for the question of early immobilization of injured areas is obvious. Unless contraindicated for other reasons, the increased encouragement to phagocytosis offered by mobility in the early hours of inflammation would seem to be desirable.

The ameboid movements of leukocytes that infiltrate the areas prove to be another factor which is potent in sweeping particulate matter against the activated mononuclear cells in the early phase of the inflammatory process. As has been stated, granulocytes enter areas of inflammation within 2 hours of the initial irritation. They moved actively about the tissues in these experiments and showed no

signs of degeneration for the periods under examination. They occurred in great numbers in all of the experimental films, *in vitro* and *in vivo*. They were absent from the control films. Phagocytosis was appreciably greater in the experimental films than in the control films in both sets of experiments (*cf.* odd numbered illustrations with companion even numbered ones).

The resting-wandering cells of the control films *in vitro*, to be sure, lacked the preliminary activation that was provided those of the experimental films. That this lack, however, does not explain their lesser degree of phagocytosis compared to that of the cells in the experimental films was proved from the experimental areas themselves. These almost always yielded a few films into which leukocytes had not yet migrated, but which contained the experimental suspensions together with activated macrophages. Phagocytosis was no more abundant in these films, however, than in the control films. Phagocytosis may well have been stimulated by virtue of opsonins and complement in the *in vitro* experiments, if not in both series, but the technical procedures *in vitro* were such that they would have functioned in experimental and control films alike, and would therefore seem irrelevant to the differences in phagocytosis in the two locations.

The play of muscular activity was in force in both control and experimental areas *in vivo* but the time of exposure varied. The time of exposure of mononuclear cells to suspensoids was held the same, on the other hand, for both control and experimental films, *in vitro*. An abundance of polymorphonuclear cells in the experimental areas, compared to their absence in the control areas, was similar in both methods of experimentation. It seems obvious, therefore, in consideration of these various facts, that the presence of the leukocytes is related to the increased degree of phagocytosis by the reticulo-endothelial cells of the experimental areas, compared to that of their control areas. The constant movement of the leukocytes over and about the mononuclear cells and suspensoids, their tendency to crawl upon the surfaces of these larger cells, and to aggregate in masses against them, obviously rubbed and pressed the suspensoids and activated mononuclear cells together again and again. This was true whether or not they themselves shared in ridding the area of the suspensoid by phagocytosis on their own part. Even when actively phagocytosing independently, as with the tubercle bacilli, they still swept particles against the mononuclear cells in their environment. These particles, in turn, adhered to the activated mononuclear cells and in due course were phagocytosed by them by the same processes of "surface phagocytosis" as those described by Wood *et al.*[5,6]

In review, then, these experiments reveal that the reticulo-endothelial cells of normal, subcutaneous tissues, *i.e.*, resting-wandering cells, share an equally, if not more important, position in early defense of the organism from invasion, or in absorption of materials injected for therapeutic purposes, than do polymorphonuclear leukocytes. They reveal that the resting-wandering cells of the area become converted to macrophages, activated in preparation for phagocytosis, and avidly phagocytic of particular matter, whether or not the polymorphonuclear cells themselves are able to cope with the substance in question. These cells become mobilized hours in advance of arrival of ameboid phagocytic mononuclear cells by way of the blood stream. Duration of exposure and size of particles determine in part the degree to which the activated cells are able to clear the areas, but of far greater significance to their ability to phagocytose the foreign particles are the movements of the underlying muscles and of the infiltrating polymorphonuclear cells. Together, these movements represent the methods by which particulate substances are rapidly brought into surface contact with the pre-existing reticulo-endothelial cells of invaded areas and quickly subjected to "surface phagocytosis."

Conclusions

The reticulo-endothelial cells of subcutaneous tissues, the so-called resting-wandering cells, represent active defenders in the first 12 hours of inflammation.

They become activated in preparation for phagocytosis by contraction, excretion of fluid from their "fluid vacuoles," and changes in the consistency of their cytoplasm.

Once activated, they are aided in making contact with particulate substances and are supported in "surface phagocytosis" by the movements of the underlying muscles and of the infiltrating ameboid polymorphonuclear cells.

They carry the burden of phagocytosis before arrival of polymorphonuclear cells; they share in the burden of phagocytosis after arrival of the polymorphonuclear cells; and they alone must phagocytose for about 12 hours the many invading substances that are not phagocytosable by the polymorphonuclear cells.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

The odd numbered figures are from the experimental areas; the even numbered, from the control areas.

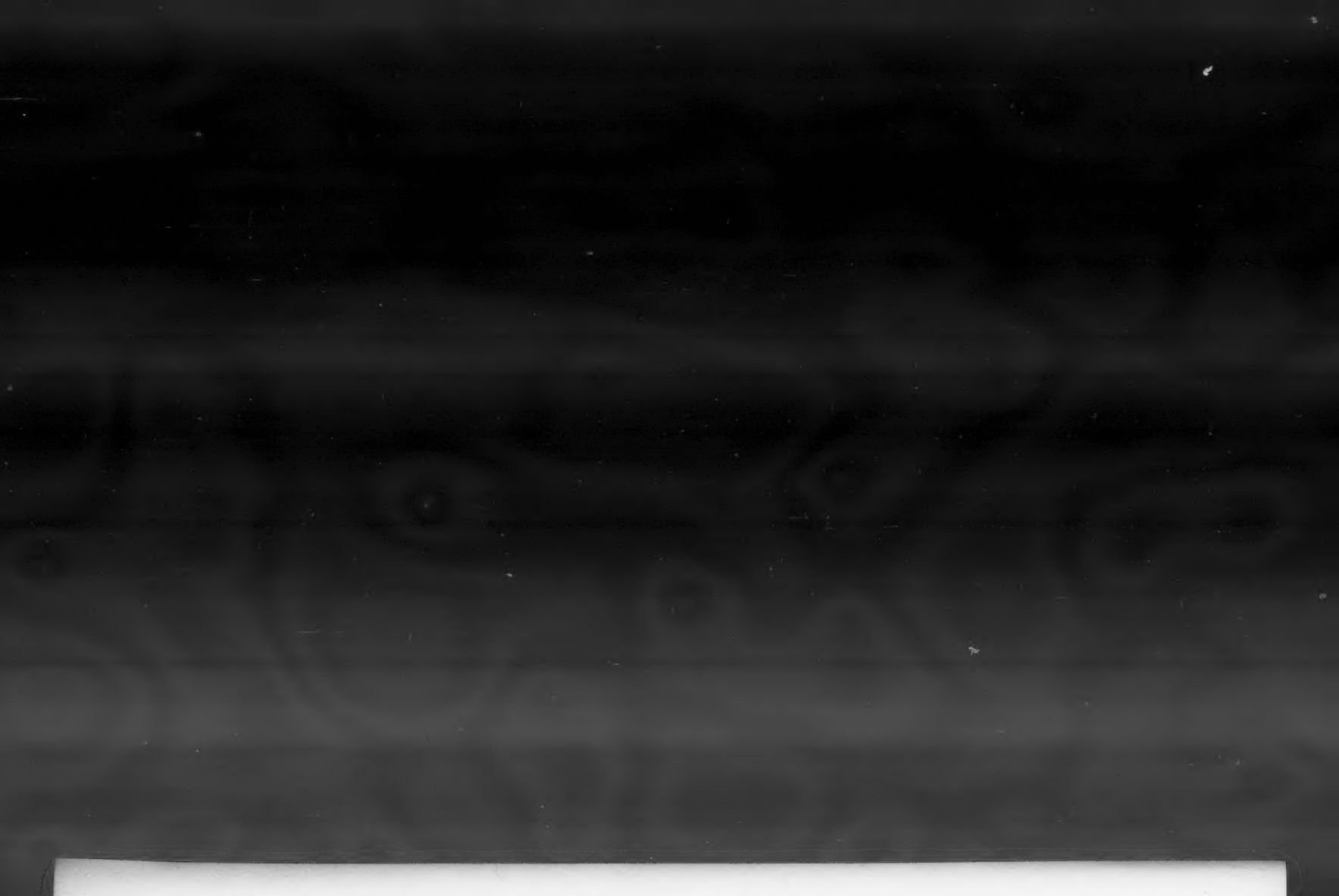
Photographs from the *in vivo* experiments are shown in Figures 1 to 8. Each set of figures is from a single mouse which had been previously injected subcutaneously, at the designated times, with the same experimental suspension. The control area was uniformly that which had been injected the more recently. Polymorphonuclear neutrophils had always invaded the experimental areas in large numbers. Few, if any, had entered the control areas.

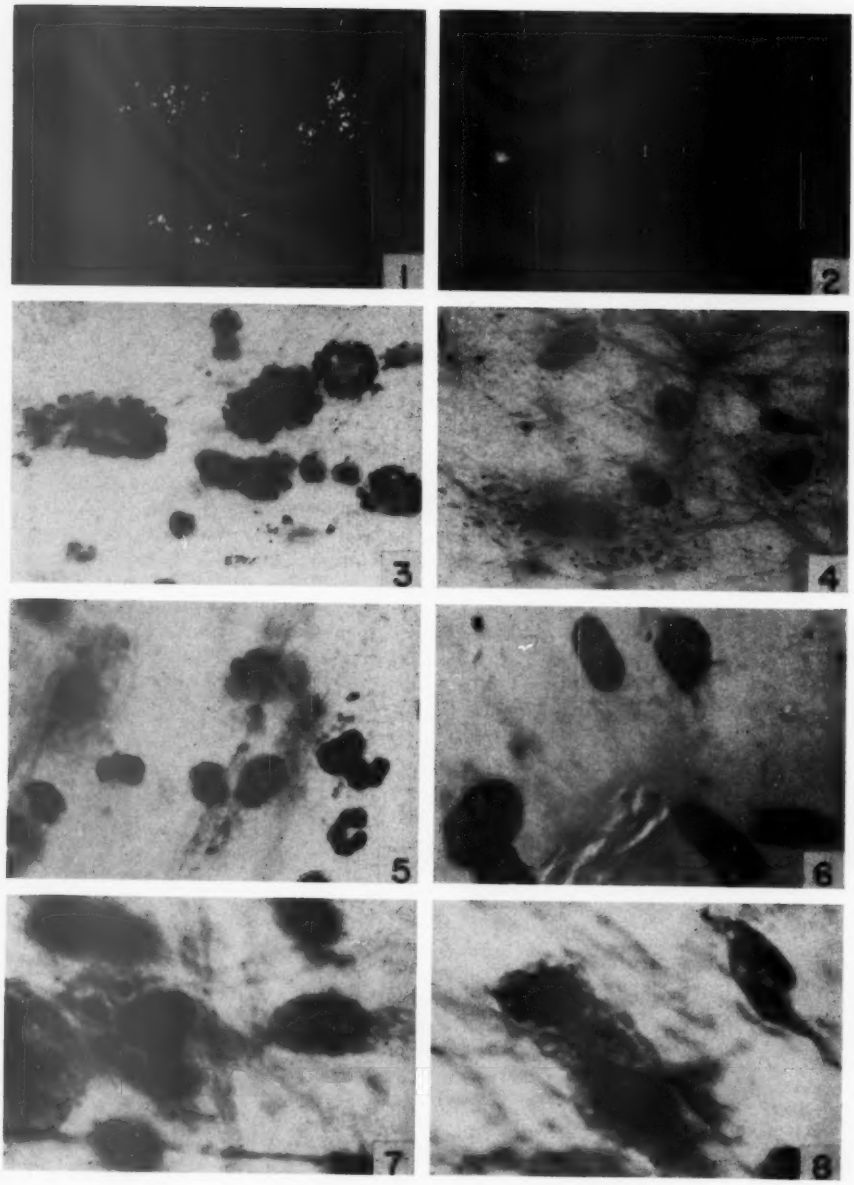
FIGS. 1 and 2. Mouse no. 27. Injected previously with the lipid, protagon, in glucose at 3 and 1 hours, respectively. Fewer reticulo-endothelial cells contained lipid in the control than in the experimental area; and it was sparse in those that did contain it, and still in the form of liquid crystals (crosses), compared to an abundance of birefringent crystals in similar cells of the experimental area. The polymorphonuclear cells did not take the lipid. Photographed with polarized light. No stain. × 900.

FIGS. 3 and 4. Mouse no. 1. Injected previously with a suspension of India ink in glucose at 5 and 2 hours, respectively. The reticulo-endothelial cells of the experimental area were all loaded with carbon as compared to a much finer deposition in similar cells of the control area. The polymorphonuclear cells did not take the carbon. Hematoxylin and eosin stain. × 500.

FIGS. 5 and 6. Mouse no. 41. Injected previously with a suspension of killed, human, tubercle bacilli in glucose at 4 and 2 hours, respectively. Many of the reticulo-endothelial cells of the experimental area contained bacilli. Fewer contained them in the control area. (Only the upper, right cell of Figure 6 contained any). Likewise the number of bacilli in a single cell was considerably greater in the experimental area. The photographs indicate merely relative numbers since changes in focal plane would have been necessary to indicate the actual number of bacilli per cell. The polymorphonuclear cells phagocytosed the bacilli in quantities. Acid-fast and methylene blue stains. × 1000.

FIGS. 7 and 8. Mouse no. 52. Injected previously with a suspension of homologous erythrocytes in glucose at 3 and 1 hours, respectively. Reticulo-endothelial cells had phagocytosed erythrocytes in both areas, but more frequently, and in greater numbers, in the experimental area. Also, often they had begun to destroy them in the experimental area, as judged by shrunken size and increased intensity of staining. Figure 7 shows an erythrophage with six red blood cells, most of which were partially disintegrated. The polymorphonuclear cells did not phagocytose the erythrocytes. Hematoxylin and eosin stain. × 1000.

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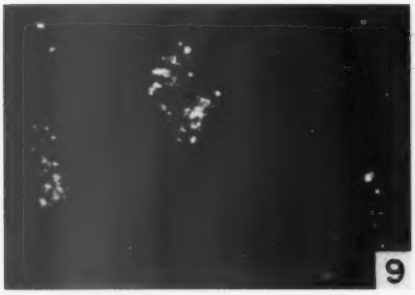
Figures 9 to 16 are photographs from the *in vitro* experiments. Each set of figures is from a single mouse. Films from the experimental and control areas were moistened with the same suspension, and incubated at 37° C. for the same period of time. The experimental areas had been injected *in vivo* 5 hours before sacrifice with 5 per cent glucose in order to assure infiltration of polymorphonuclear cells. The control films were from normal, uninjected areas.

FIGS. 9 and 10. Mouse no. 70. Films moistened with the lipid, protagon, in serum-glucose (4:1). There was practically no phagocytosis of the lipid by the reticulo-endothelial cells of the control area. The suspended particles that had been injected merely glowed diffusely in polarized light. Phagocytosis was general on the part of similar cells in the experimental area, on the other hand, and free particles were too sparse to show in polarized light. There was no evidence of phagocytosis by the polymorphonuclear cells. Photography with polarized light. No stain. × 900.

FIGS. 11 and 12. Mouse no. 21. Films moistened with a suspension of India ink in serum. Phagocytosis by the reticulo-endothelial cells of the experimental area was generalized, though of considerably less degree than on the part of similar cells from the experimental area *in vivo* (Fig. 3), which was injected with India ink. Carbon tended merely to rim the phagocytic vacuoles in this experiment whereas it filled the vacuoles homogeneously in the latter experiment. The reticulo-endothelial cells of the control area had phagocytosed merely sporadically and to much less degree. Carbon was not taken up by the polymorphonuclear cells. Hematoxylin and eosin stain. × 500.

FIGS. 13 and 14. Mouse no. 43. Films moistened with a suspension of killed, human tubercle bacilli in serum-Locke's solution (1:1). The reticulo-endothelial cells of the experimental area frequently contained several to many bacilli, whereas those of the control area often were free of bacilli or contained a maximum of three or four. These findings parallel those obtained *in vivo* with the bacilli, but in much lesser degree (Fig. 5). The polymorphonuclear cells in the experimental area also phagocytosed bacilli vigorously. Acid-fast technique and methylene blue stain. × 1000.

FIGS. 15 and 16. Mouse no. 50. Films moistened with a suspension of homologous erythrocytes in serum-Locke's solution (1:1). No evidence was obtained of erythrophagocytosis by the reticulo-endothelial cells of the control area. On the other hand, phagocytosis of one to five erythrocytes had occurred sporadically by the reticulo-endothelial cells of the experimental area, though much less frequently than in the *in vivo* experiments with similar suspension. No evidence was observed of erythrophagocytosis by the polymorphonuclear cells. Hematoxylin and eosin stain. × 1000.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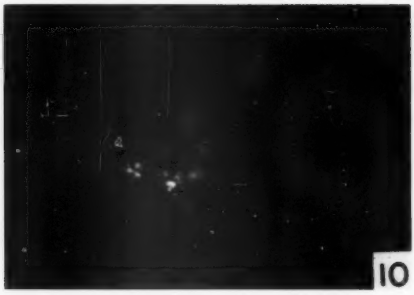
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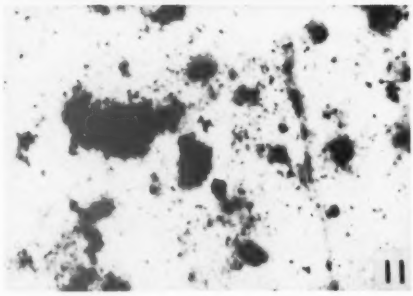
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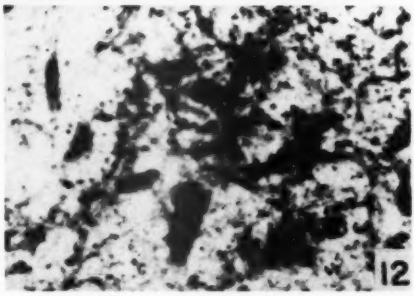
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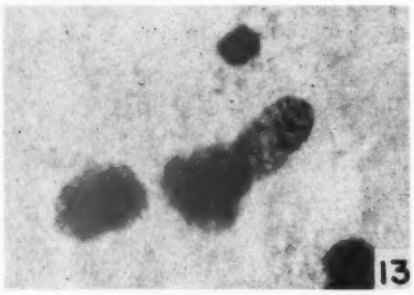
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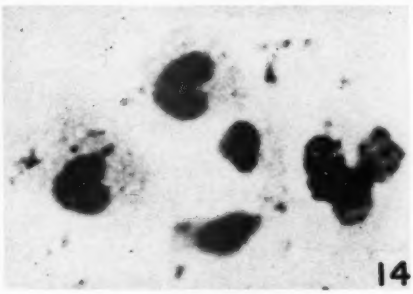
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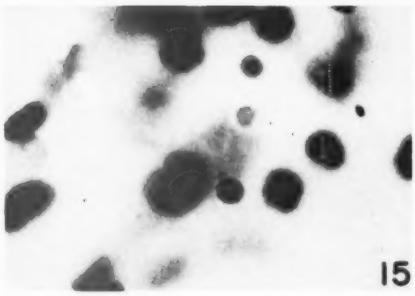
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THE EFFECT OF METHIONINE IN EXPERIMENTAL WOUND HEALING
A Morphologic Study *

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The morphologic and functional aspects of connective tissue have become problems of major interest in the last decade. The work by Klemperer and his associates[1,2] has been particularly important, both in defining the structural characteristics of collagen diseases and in contributing a general hypothesis on the unity of the connective tissues.

Many factors control the metabolism and formation of connective tissue. Among the endogenous factors, hormones and enzymes have been studied extensively although much data are still lacking.[3,4] Less attention has been paid to the rôle of exogenous factors in the intimate physiologic activity of connective tissue. With the exception of ascorbic acid,[5,6] it can be said that little is known of the effect of the diet on the synthesis and maintenance of mesenchymal elements.

The effect of dietary protein on the connective tissue as studied in wound healing has received some attention. Depletion of plasma or tissue protein has been shown clinically[7-10] and experimentally[11-18] to prolong the time of wound healing. Several hypotheses have been offered in explanation: (a) lowering of the colloid osmotic pressure of the plasma with resultant edema[13]; (b) lack of raw material for building tissues[14]; and (c) absence of essential amino acids.[14] The last hypothesis is of interest since it points to the possible influence of specific protein components in the mechanism of wound healing.

The effect of one specific protein component on the tensile strength of healing wounds of protein-deficient rats was studied by Localio, Morgan, and Hinton.[19] They chose methionine because it is an essential amino acid, because it spares nitrogen in animals, and because it contains a sulfhydryl radical. It was found that protein depletion decreases the tensile strength of experimental wounds. However, after the administration of dl-methionine to protein-depleted animals, the tensile strength of the wound was equal to that of the normal animals. In explanation, Localio and associates suggested that the sulfhydryl radical may be deficient and not readily available to the wound of the

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† Kellogg Fellow in Pathology from the Instituto Nacional de Cardiologia, Mexico City, Mexico.

protein-deficient rat, and that deficient —SH enzyme activity may be one of the reasons for delayed healing.

Whereas Localio *et al.*[19] withheld protein for a period of 5 weeks, Williamson, McCarthy, and Fromm[20] obtained essentially the same results after a low protein diet of 5 days' duration. Also, it was found that the rate of healing was related to sulfur supplied by protein rather than to the nitrogen supplied by the protein.

Morphologic studies of wounds were not made by Localio *et al.*[19] or by Williamson and his associates.[20] We consider it to be of some interest to determine the morphologic changes responsible for the differences in tensile strength. This study is primarily a morphologic analysis of experimental wound healing under a short-term protein deficiency supplemented by methionine.

Material and Methods

Forty-eight male albino rats weighing 100 to 150 gm. were used. After 1 week of observation for weight gain, the rats were divided into four groups and given different diets as shown in Table I.

Table I

*Composition of Diets per 100 gm.**

	I	II	III	IV
Casein	20	0	0	0
Methionine	0	0	0.147	0
Valine	0	0	0	0.147
Sucrose	63	83	82	82

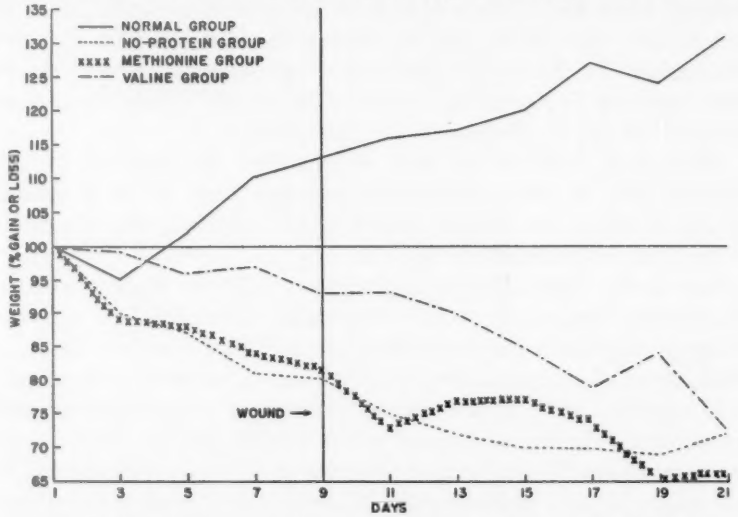
* All diets contained the following elements per 100 gm.: lard, 10 gm.; corn oil, 2 gm.; salt mixture,[21] 5 gm.; thiamine hydrochloride, 1 mg.; riboflavin, 1 mg.; pyridoxine hydrochloride, 1 mg.; calcium pantothenate, 4 mg.; nicotinic acid amide, 15 mg.; inositol, 5 mg.; 2-methyl naphthoquinone, 0.5 mg.; choline chloride, 25 mg.; vitamin A, 1500 international units; vitamin D, 210 I.U.

Group I (normal control) was given a complete basic diet. Group II (no-protein) was given a basic diet without protein. Group III (methionine group) was given no protein; supplemental methionine was given in the same amount as used by Williamson *et al.*[20] Group IV (valine control) was given neither protein nor methionine. The purpose of this group was to test the possible effect of sulfur in methionine. Valine was selected because it contains no sulfur, because it is also an essential amino acid, and because it has the same nitrogen value as methionine.

All animals were given 15 gm. of the diet daily, and water *ad libitum*. Although no special attempt was made to determine accurately the amount of diet consumed daily by each group, the normal control

group was the only one which ate the complete amount with any regularity.

Wounds were made by excising from the back with scissors a circular area of skin and subcutaneous tissue measuring 6 to 8 mm. in diameter. The wounds were not closed. After shaving, the skin was wiped with 70 per cent alcohol. Although no additional antiseptic measures were taken, there were only three obviously infected wounds in the entire group. These animals are not included in the series. Representative animals from each group were sacrificed 2, 4, 6, 8, 10, and 12 days after the wounds were made. At that time, the wound was excised with a wide margin of normal skin, fixed for 24 hours in 4 per cent basic lead acetate, washed for 24 hours in tap water, and fixed in 10 per cent formalin for 24 hours. Two or more blocks were taken from each wound, embedded, and cut at 4 μ. Histologic methods were



Text-fig. 1. Average change in weight of animals in each dietary group, shown graphically as percentage gain or loss in reference to the initial weight.

selected to give a fairly complete picture of all elements of the connective tissue. Stains used were hematoxylin and eosin, toluidine blue, thionine, a combination of the Verhoeff and van Gieson stains, and Wilder's method for reticulum. The slides were first examined without knowledge of the group to which they belonged. They were then rearranged and examined for specific differences within the various groups.

Results

The striking differences in the general appearance of the rats from the different groups are reflected in the weight chart (Text-fig. 1).

Animals in the normal control group were sleek and agile; they were easily handled. The consistent weight gain within this group is an indication of their appearance; wound healing was rapid and usually was completed by 8 to 10 days.

Animals in the no-protein and methionine groups rather rapidly developed rough hair, showed inhibition of activity, and were more irritable during handling. The consistent and parallel weight loss of both groups is an indication of the general appearance of these two groups. The wounds of the no-protein group healed poorly; they were incompletely healed at the termination of the experiment. Whereas the animals in the methionine group had the general appearance of those in the no-protein group, the wounds healed as rapidly and completely as those in the normal control group. Animals from the valine group showed more individual variation than animals from the previous groups. There was a fairly consistent but characteristically irregular weight loss. Although systematic observations were not made, it is our impression that the general appearance was somewhat better than in the no-protein or methionine groups. The wounds in this group were unhealed at the termination of the experiment.

Microscopic observations will be described for each of the six different dates at which the animals were sacrificed. To avoid tedious repetition, discussion will be limited to the differences found. Additional data can be found in Table II.

Two Days. Forty-eight hours after the wound was made, the histologic picture was mainly that of edema with remarkably few mononuclear or polymorphonuclear leukocytes. Toluidine blue and thionine stains revealed a large amount of metachromatic material in all groups. Inflammatory cells present were mononuclear macrophages and lymphocytes. In the normal and methionine groups, the thickness of the areas of reaction and edema was greater and more regular than in the no-protein and valine groups. This was the only observed difference.

Four Days. At 4 days the normal group showed a beginning proliferative reaction in an area in which a few mononuclear macrophages and a little edema remained. A small amount of metachromatic material was seen. A moderate number of immature fibroblasts were present mainly deep in the wound, and there was early capillary proliferation (Fig. 1). In contrast, the no-protein group showed less proliferation. Immature fibroblasts were fewer and capillaries were minimal in number. Edema was decreased; only a few lymphocytes and macrophages were found (Fig. 2). The amount of metachromatic material was equal to that seen in the normal group. The methionine

TABLE II

Summary of Microscopic Features of Wound Healing in the Four Experimental Groups

	2 days				4 days				6 days				8 days				10 days				12 days			
Diet*	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
Edema†	3+	2+	2+	3+	2+	1+	o	1+	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Leukocytes	o–1+	o–1+	o–1+	o–1+	o–1+	o	1+	1+	o	o	o	o	o	1+	o	1+	o	o	o	o	o	o	o	o–1+
Macrophages	1+	1+	1+	1+	2+	2+	2+	2+	o–1+	1+	o–1+	o–1+	o–1+	1+	o	o–1+	o	o–1+	o	o–1+	o	o–1+	o	1+
Fibroblasts	o	o	o	o	2+	1–2+	2+	2+	3+	2+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Reticulum fibers	o	o	o	o	o–1+	o	o	o	2+	1+	2+	1+	2+	2+	2+	2+	3+	3+	3+	3+	3+	3+	3+	3+
Collagen fibers	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o–1+	o	o–1+	o	2+	1+	2+	1+
Elastic fibers	o	o	o	o	1+	1+	o	o	o	o	o	1+	1+	1+	1+	2+	1+	1+	1+	o	2+	2+	2+	2+
Metachromatic material	3+	3+	3+	3+	1+	1+	1+	1+	o	1+	o	o	o	o	o	o	o	o	o	o	o	o	o	o
Capillaries	o	o	o	o	2+	o–1+	2+	o–1+	3+	2+	3+	2+	3+	2+	3+	3+	2+	3+	1+	3+	2+	3+	1–2+	2–3+

* Group I, normal control group; group II, no-protein group; group III, methionine group; group IV, valine group.

† o = none; 1+ = slight; 2+ = moderate; 3+ = advanced.

group differed from the normal group only in that proliferation was more irregular in depth (Fig. 3). The wounds were distinctly different from those of the no-protein group. The valine group was comparable to the no-protein group except that the area of reaction was greater in depth. However, the number of fibroblasts was very small and only a few scattered capillaries were seen. The amount of metachromatic material was the same as in the other groups.

Six Days. The normal group at 6 days showed considerable progression of healing since the previous period. There was almost no edema; very few lymphocytes or mononuclear macrophages were seen. The wound was now composed of many mature and immature fibroblasts with an intense degree of capillary proliferation (Figs. 4 and 5). Some reticulum fibers appeared for the first time (Fig. 6). They were arranged along the lines of stress. No metachromatic material was seen. The wounds from the no-protein group showed a distinctly different picture and were much less mature. Slight edema with scattered mononuclear macrophages (Fig. 7) and a small amount of metachromatic material were still present. Fibroblasts were fewer than in the normal group. They were also less mature (Fig. 8). Scattered argyrophilic fibers showed no orientation (Fig. 9). Only occasional capillaries were seen. Wounds from the methionine group differed from those of the normal group in that the thickness of the zone of reaction was slightly less marked (Figs. 10, 11, and 12). However, the valine group showed much less progression of healing and was again comparable to the no-protein group. The area of granulation tissue was irregular in this group. No mononuclear macrophages or polymorphonuclear leukocytes remained and fibroblasts found in moderate numbers showed no orientation. Capillary proliferation was about equivalent to that of the no-protein group.

Eight Days. The normal group showed an advanced stage of healing 8 days after the wounding. Mature, oriented fibroblasts with thick, straight, abundant reticulum fibers were arranged in parallel lines. These lines were broken only by capillaries which had thick walls (Figs. 13, 14, and 15). Scattered bundles of fibers had a yellowish cast with the Verhoeff-van Gieson stain, which was interpreted as evidence of early deposition of collagen. The differences between the normal and no-protein groups were now greater. Although the no-protein group showed a large number of fibroblasts, they were immature and lacked any particular arrangement. The reticulum fibers likewise showed no tendency to parallel arrangement. Capillaries were less numerous than in the normal group and there was no evidence of collagen formation (Figs. 16, 17, and 18). At this time, the wounds from

the methionine group were histologically identical with those of the normal group. A similar amount of collagen was present (Figs. 19, 20, and 21). The valine group was also roughly similar to the no-protein group. However, capillaries were more numerous in the valine group and there was a slight tendency to parallel arrangement of reticulum fibers.

Ten Days. At 10 days the normal group showed a well healed wound which lacked only maturation of fibers. All of the fibroblasts appeared as thin, elongated cells with oriented, closely packed reticulum fibers and dark nuclei (Fig. 22). With the reticulum stain, a slight amount of collagen could now be detected. Capillaries were fewer than at 8 days. Short, thick-walled capillary segments were seen. The appearance of wounds from the methionine group was similar to those of the normal group (Fig. 24). Although there was progression of healing in the no-protein group since the 8-day period, the wounds remained immature. There was a mixture of mature and immature fibroblasts. Numerous reticulum fibers were thin and showed no organization. There was very little collagen. Long, delicate capillaries remained (Fig. 23). The valine group differed little from the no-protein group.

Twelve Days. At 12 days wounds from the normal (Fig. 25) and methionine (Fig. 27) groups showed complete healing and were indistinguishable. Thickness of fibers was the only feature which distinguished the wound area from the adjacent dermis. We were unable also to distinguish between the no-protein (Fig. 26) and valine groups at this period. Wounds from the no-protein and valine groups had progressed little from the previous date. They showed a mixture of mature and immature fibroblasts. In spite of this manifestation of immaturity, reticulum fibers were abundant and a few collagen fibers were seen. Organization of fibers was poor. Many capillaries still remained.

Discussion

Our results offer an adequate histologic explanation for the decreased tensile strength reported in wounds in animals fed protein-free diets. When compared to wounds from animals given a normal diet or supplemental methionine, wounds from the no-protein groups show a more narrow zone of reaction, fewer capillaries and fibroblasts, and a delay in the formation of fibers. It is evident that these factors will result in a scar with decreased tensile strength. Although our animals were kept on protein-deficient diets for short periods of time, it is probable that a similar morphologic explanation would obtain for protein deficiency of longer duration; under these experimental conditions,

methionine has been shown to exert the same beneficial effect on the tensile strength of healing wounds.[19] Furthermore, it is apparent that methionine exerts a definite effect on connective tissue. Under the influence of this essential amino acid, proliferating connective tissue elements mature at a normal rate. They will form a scar equal in both structural and functional aspects to that from a normal animal. The observations made in this experiment, together with the results of experiments on tensile strength, indicate that methionine plays an important rôle in the normal mechanism of the synthesis of connective tissue.

As indicated before, several hypotheses have been offered to explain the rôle of protein in wound healing. From our results and the results from experiments determining tensile strength, we conclude that the effect of protein is determined by its methionine content. Since normal wound healing occurs when methionine is given as the only amino acid source, methionine will logically account for the beneficial effect of protein on wound healing. That the improved healing in the methionine group may be due to a non-specific effect from the added amino acid is excluded by the valine group in which no beneficial effect was found. Additional support for the specific effect of methionine is obtained from the weight chart (Text-fig. 1); although the weight losses of the methionine and no-protein groups were equal, the methionine group healed as well as the normal group. Thus, other factors remaining constant, it is probable that protein deficiency means methionine deficiency in regard to wound healing.

The mechanism by which methionine exerts this specific effect on connective tissue cannot be elucidated from our observations. Two broad possibilities should be considered: (a) Methionine acts "at a distance" on wound healing; (b) methionine is utilized locally by the proliferative elements of the area of wound healing. With regard to the distant action of methionine, Brush, Willman, and Swanson[22] have expressed the opinion that methionine "does not act in the general maintenance of body tissues but in the synthesis of functional proteins and important metabolites." It is also well known that methionine has a nitrogen-sparing action in normal or wounded animals.[23-26] Further, Williamson and associates[20] found that wounded rats fed a protein of very low biologic value (gelatin) plus methionine would show a rate of healing comparable to normal animals. They concluded that methionine increases the efficiency of utilization of either dietary or tissue protein, or both. Therefore, it is possible that the effect of methionine on connective tissue is simply to increase the rate of utilization of available protein.

The possibility that methionine is partly or wholly utilized locally at the area of wound healing has been suggested by Localio and associates[19] and by Williamson and collaborators.[20] These authors considered that the sulfur radical present in methionine is utilized at the wound as either sulfhydryl radicals or as sulfate. This last suggestion is based on the findings of Sylvén[27] and Layton[28] that granulation tissue has a high sulfur content; they believed that this sulfur is present as chondroitin sulfate. Metachromasia is considered to be an index of chondroitin sulfate.[29] Since we have observed no differences in the degree of metachromasia in our animals, it may be possible that methionine does not participate in the formation of chondroitin sulfate. However, it is doubtful that fine differences in the amount of sulfated acid mucopolysaccharides will be shown by either toluidine blue or thionine stains. Thus, the possibility that the sulfur of methionine is used locally in the area of wound healing for the synthesis of chondroitin sulfate remains.

It has been suggested that the ground substance has a cellular origin. Both mast cells[26,30] and fibroblasts[31,32] have been implicated. Our observations support neither view, since very few cells were present at the time when the metachromatic material was most abundant. In a study of experimental syphilis in the rabbit testis, Scott and Dammin[33] also found metachromatic material in the interstitial tissue before a cellular infiltrate appeared. Contrary to Sylvén,[27] we did not find a decrease in the number of mast cells at the time when metachromasia became apparent. Further, fibroblasts began to appear at a time when metachromasia was decreasing. Practically the only cells present at the time when metachromatic material was abundant were mononuclear macrophages and polymorphonuclear leukocytes. Among the possible explanations, two seem more likely: (1) Ground substance was there and became apparent through a physical or chemical change[34]; and (2) ground substance is deposited with edema fluid by filtration.

Summary and Conclusions

The effect on experimental wound healing of a short-term protein deficiency supplemented by methionine has been studied in the rat. Animals given a normal diet, a protein-deficient diet, and a protein-deficient diet supplemented by valine were used as controls. It was found that protein deficiency will delay the rate of wound healing; when methionine is added to a protein-deficient diet, the morphologic pattern of wound healing returns to normal. Thus, methionine is capable of exerting the same beneficial effect as protein in wound healing. The addition of valine to the protein-deficient diet was without effect.

It is concluded that methionine plays an important rôle in the synthesis of the connective tissues. The possible mechanisms by which methionine exerts this effect are discussed.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Normal group, 4 days after wounding. Early capillary proliferation with numerous immature fibroblasts. Few inflammatory cells remain. The dark areas surrounding the walls of the newly formed capillaries correspond to metachromatic material. Toluidine blue stain. × 125.

FIG. 2. No-protein group, 4 days after wounding. Edema and inflammatory cells predominate. No capillaries are seen. The area of reaction is smaller as compared with Figure 1. Toluidine blue stain. × 125.

FIG. 3. Methionine group, 4 days after wounding. Capillaries and immature fibroblasts are present to the same degree as in Figure 1, but the area of reaction is smaller. Toluidine blue stain. × 125.

FIG. 4. Normal group, 6 days after wounding. Abundant capillaries and early arrangement of cells and fibers in parallel layers. No inflammatory cells are seen. Toluidine blue stain. × 95.

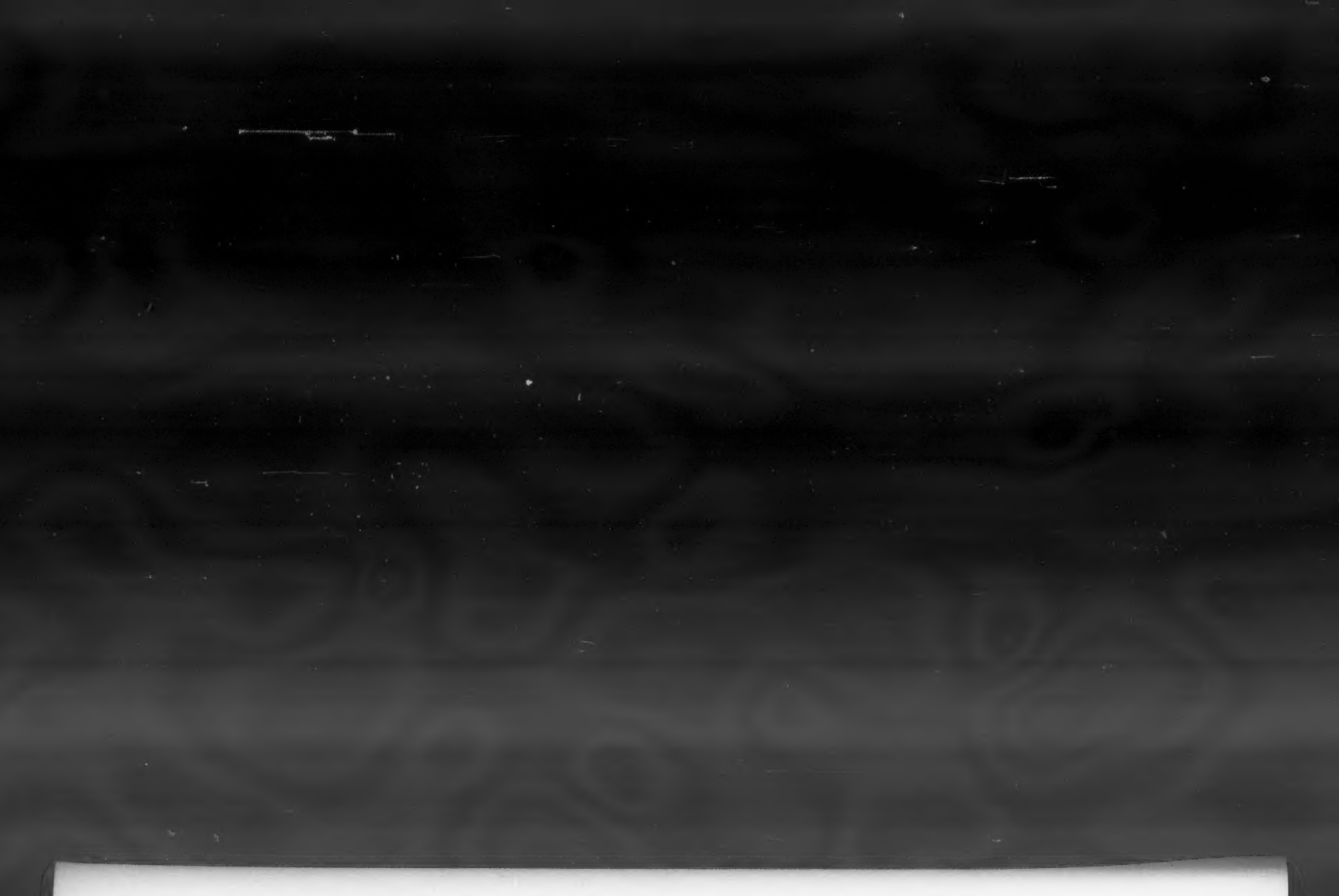
FIG. 5. High-power view of Figure 4. Immature, rounded fibroblasts are oriented in parallel planes. Verhoeff-van Gieson's stain. × 485.

FIG. 6. Normal group, 6 days after wounding. Reticulum fibers showing tendency to orientation in horizontal planes. Wilder's reticulum stain. × 120.

FIG. 7. No-protein group, 6 days after wounding. Thin area of reaction. Edema and inflammatory cells are still present. Very few capillaries. Toluidine blue stain. × 95.

FIG. 8. No-protein group, 6 days after wounding. Mixture of immature fibroblasts and inflammatory cells. There is no tendency to orientation. Verhoeff-van Gieson's stain. × 485.

FIG. 9. No-protein group, 6 days after wounding. Few short, thick reticulum fibers with no tendency to arrangement in parallel planes. Wilder's reticulum stain. × 120.



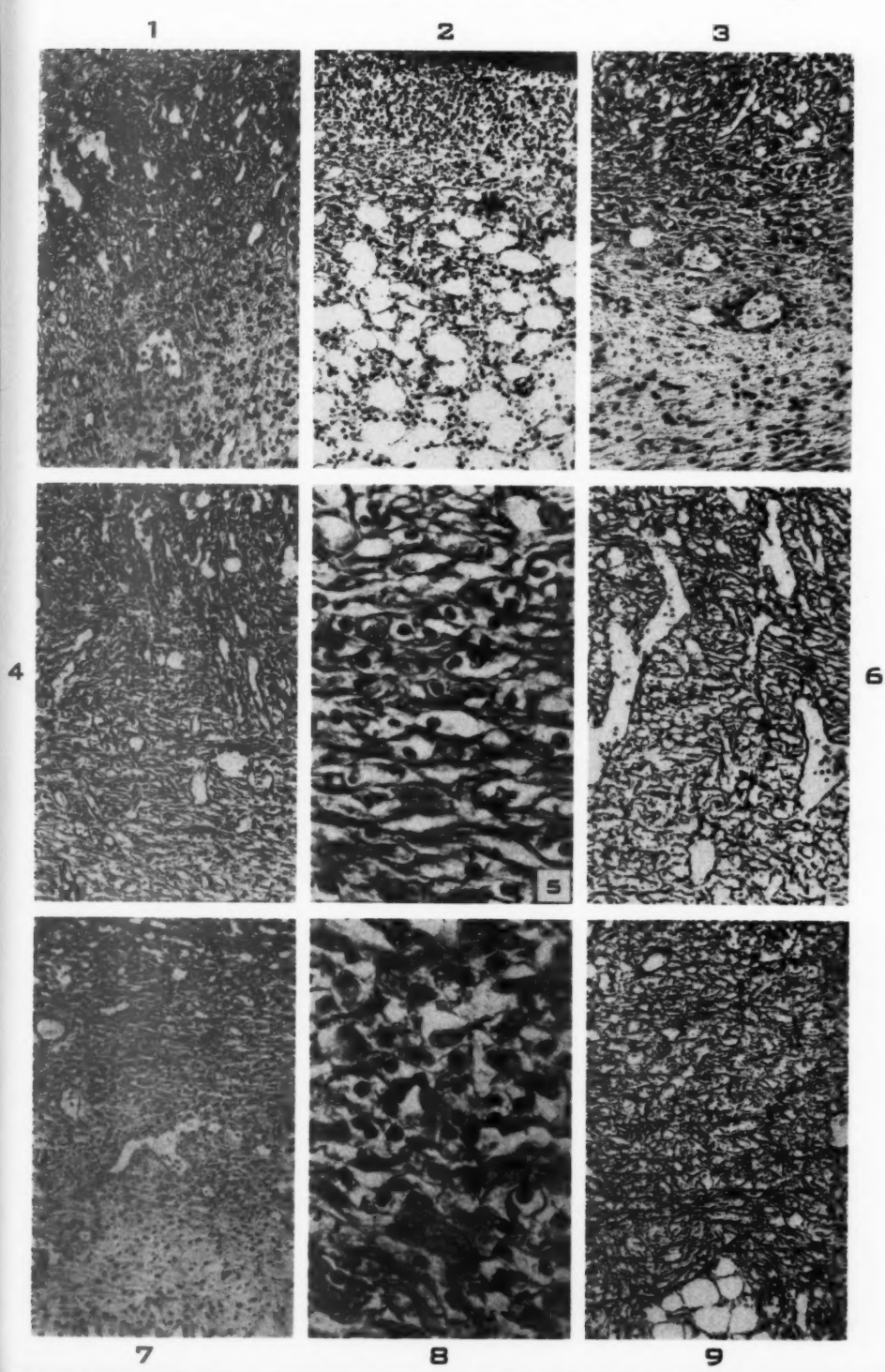

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FIG. 10. Methionine group, 6 days after wounding. Abundant capillaries and fibroblasts with definite orientation. Toluidine blue stain. × 95.

FIG. 11. Methionine group, 6 days after wounding. Immature fibroblasts arranged in parallel planes. Verhoeff-van Gieson's stains. × 485.

FIG. 12. Methionine group, 6 days after wounding. Reticulum fibers showing a tendency to orientation in parallel planes. Wilder's reticulum stain. × 120.

FIG. 13. Normal group, 8 days after wounding. The number of capillaries has decreased and fibroblasts and fibers show a more compact pattern. Toluidine blue stain. × 95.

FIG. 14. Normal group, 8 days after wounding. Mature and a few immature fibroblasts. Verhoeff-van Gieson's stain. × 485.

FIG. 15. Normal group, 8 days after wounding. Abundant reticulum fibers showing orientation in parallel planes. Wilder's reticulum stain. × 120.

FIG. 16. No-protein group, 8 days after wounding. The area of reaction is very thin. There is a mixture of inflammatory cells and immature fibroblasts without any definite pattern of arrangement. Toluidine blue stain. × 95.

FIG. 17. No-protein group, 8 days after wounding. Mixture of immature fibroblasts and a few lymphocytes without arrangement. Verhoeff-van Gieson's stain. × 485.

FIG. 18. No-protein group, 8 days after wounding. Thin, sparse reticulum fibers arranged in horizontal planes. Wilder's reticulum stain. × 120.

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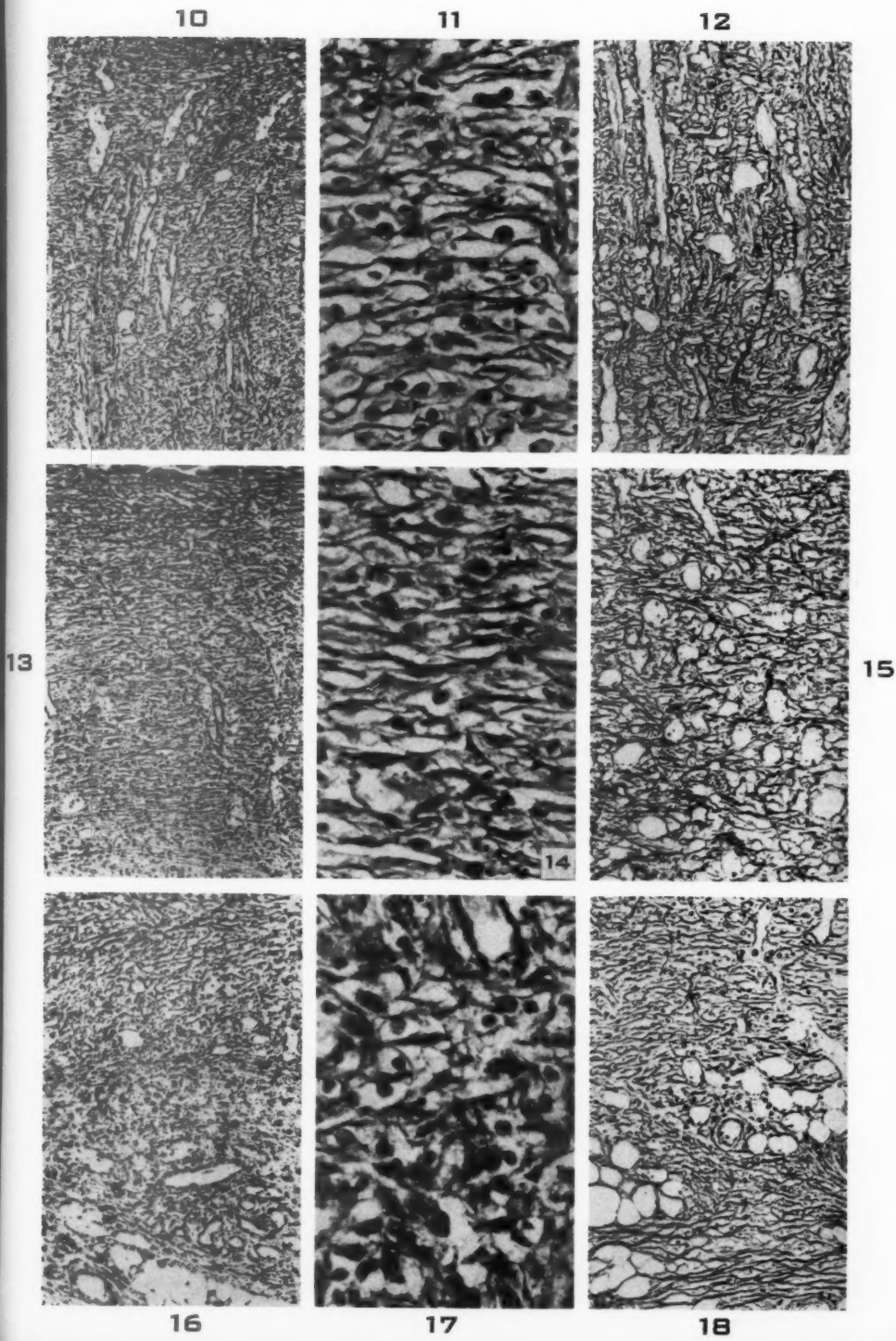
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FIG. 19. Methionine group, 8 days after wounding. Mature fibroblasts and a few inflammatory cells. The orientation in parallel planes is apparent. Toluidine blue stain. × 95.

FIG. 20. Methionine group, 8 days after wounding. Mature and immature fibroblasts. Verhoeff-van Gieson's stain. × 485.

FIG. 21. Methionine group, 8 days after wounding. Thick, wiry reticulum fibers with some branching. Wilder's reticulum stain. × 120.

FIG. 22. Normal group, 10 days after wounding. Well organized scar with a few capillaries. Occasional fibers show some of the characteristics of collagen. Toluidine blue stain. × 95.

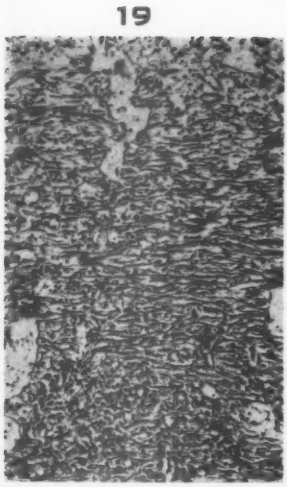
FIG. 23. No-protein group, 10 days after wounding. The area of reaction is thin. There are some inflammatory cells and numerous capillaries. Most of the fibroblasts are immature and show no arrangement. Toluidine blue stain. × 95.

FIG. 24. Methionine group, 10 days after wounding. Essentially the same as Figure 22, with the exception of a few inflammatory cells. Toluidine blue stain. × 95.

FIG. 25. Normal group, 12 days after wounding. The scar shows a more compact arrangement and the number of collagen fibers is increased as compared to Figure 22. Toluidine blue stain. × 95.

FIG. 26. No-protein group, 12 days after wounding. Loose, vascular granulation tissue with numerous inflammatory cells. For comparison with Figure 4. Toluidine blue stain. × 95.

FIG. 27. Methionine group, 12 days after wounding. The wound differs from that in Figure 25 only in the size of the area of reaction. Toluidine blue stain. × 95.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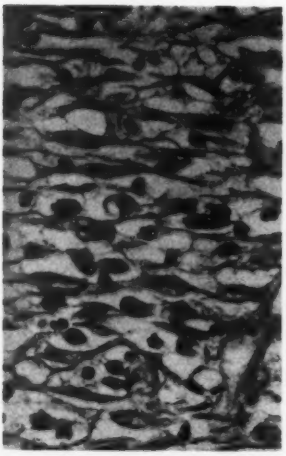
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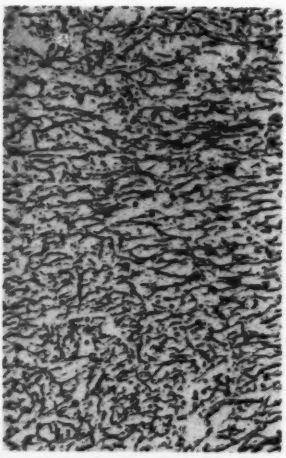
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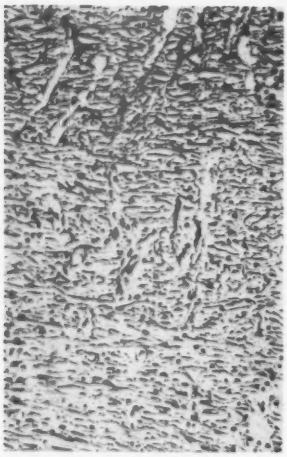
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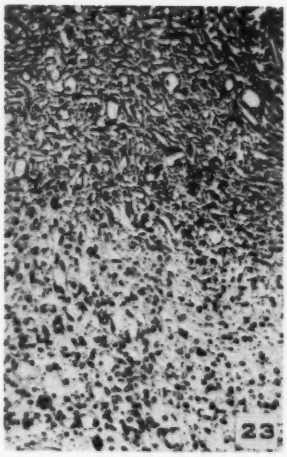
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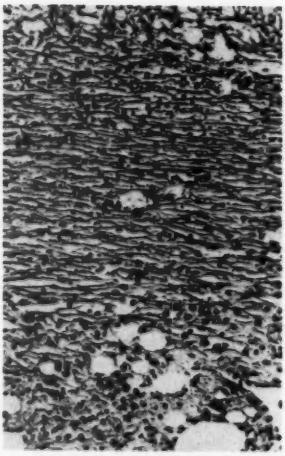
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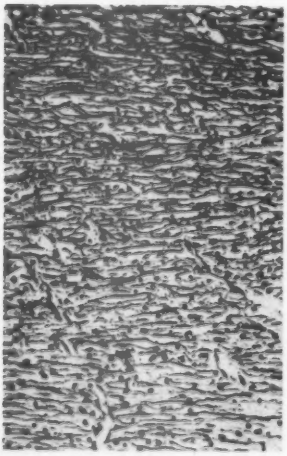
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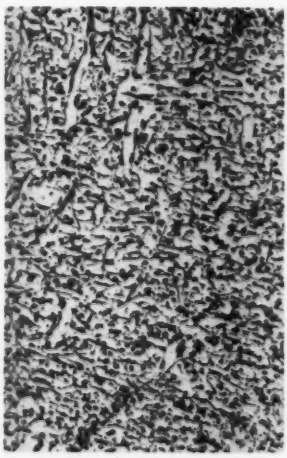
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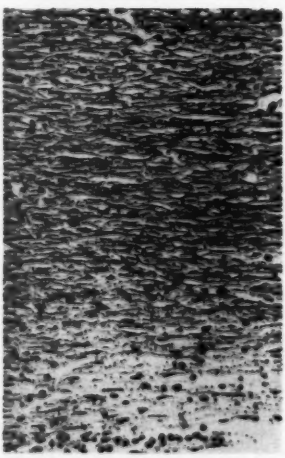
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THE BRONCHOPULMONARY VENOUS COLLATERAL CIRCULATION WITH SPECIAL REFERENCE TO EMPHYSEMA *

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Numerous anatomical and pathologic studies in various laboratories have yielded a partial elucidation of the form and function of the arterial side of the double vascular supply of the lungs.[1-9] With fibrosing pulmonary disease, the collateral blood supply of the lungs gains in relative importance. In part, this results from obliteration of the pulmonary arterial and capillary bed; but the bronchial arterial system may become immensely expanded. Concomitantly, large precapillary anastomoses appear between the two arterial circulations, where the high pressure systemic circulation is in direct communication with the low pressure pulmonary circulation.[7,10] Consequently, desaturated blood in the pulmonary artery is shunted away from the diseased parenchyma. Furthermore, these communications represent points of resistance, that, when numerous enough, can contribute to pulmonary arterial hypertension. It has even been shown that through them blood can be forced from the systemic side into the pulmonary artery, backwards toward the hilum against the usual direction of the stream.[9] The quantitative factors of flow are difficult to analyze amid the complexities attending chronic disease of the lung, but in experimental dogs, after ligating the pulmonary artery, the collateral flow approaches one third of the right cardiac output.[11] This immense flow is a burden entirely upon the left side of the heart. A diagram of the interrelationships of the two circulations is reproduced in Text-figure 1.

Less well understood are the interrelations and even the form of the venous systems of the lungs. For purposes of this discussion the term bronchopulmonary will be used for veins whose immediate function is the drainage of bronchi or bronchioles, and the interstitial tissues of the lung. The most thorough anatomical study that has been made is still that of Zuckerkandl[12] (1882) who injected the bronchopulmonary venous system with Berlin blue from the hilum, and also, so to speak, "through the back door," from the pulmonary venous side. He found, as had others before him, that the capillaries of the smaller bronchi and bronchioles are gathered into venules that drain directly into

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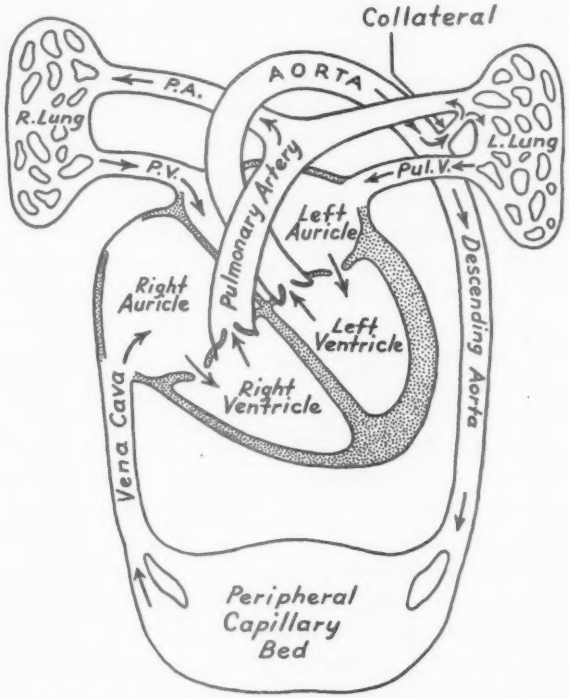
pulmonary veins, but that the proximal two or three orders of branches of the trachea are drained by vessels that he classified into three groups:

1. Recurrent: branches of the pulmonary veins that sweep in recurrent fashion proximally.

2. Anterior bronchial veins that drain the anterior surfaces of the lungs, emptying on the right side into the azygos system and on the left into the posterior bronchial venous system.

3. Posterior bronchial veins that drain the posterior surfaces of the bronchi. Those of the right side enter the upper end of the azygos vein, and those on the left drain into the accessory hemi-azygos vein or



Text-fig. 1. In this diagram it is assumed that all segments of the left lung are involved in bronchiectasis and that a large arterial collateral circulation has been built up. For convenience this is pictured as a single vessel anastomosing with the main pulmonary artery. Actually the anastomoses would be innumerable and would be located rather distally in the walls of the sacs (third order divisions of the segmental bronchi and beyond). The bronchial arterial supply of the normal right lung can be ignored. It is obvious that the blood arriving via the collateral channels and traversing the pulmonary capillaries travels in a circle involving only the left side of the heart. An alternative course for a portion of the collateral flow is into the pulmonary artery toward the hilum, impelled by the higher pressure on the aortic side of the anastomosis. The venous side of the collateral circulation is considered in Text-figure 2.

rarely into the innominate veins. Most important is Zuckerkandl's description of the large and abundant communications of all of these bronchial venous systems with pulmonary veins. The larger of them communicate with major pulmonary veins by means of grossly visible stomata. Some of these bronchial veins may drain directly into the left auricle.

Miller[13] described the venous drainage of the distal portions of the respiratory tree into the pulmonary veins, but made only the most general statements concerning the drainage of the proximal orders of bronchi into the systemic venous vessels.

A somewhat different account from that of Zuckerkandl[12] was given by Marchand, Gilroy, and Wilson[14] from their recent study of the pulmonary vasculature with a vinylite corrosion method. They classified the draining vessels of the bronchi into (1) deep or true bronchial veins, and (2) pleurohilar veins. They reported tracing the former from the respiratory bronchioles proximally, first as a plexus and then as a conjoint single trunk, for each lobar bronchus, that enters a large pulmonary vein. These vessels doubtless correspond to the peripheral bronchopulmonary system and "recurrent" branches described by Zuckerkandl. The pleurohilar vessels were described as draining the pleural and hilar structures into the azygos system. Numerous intercommunications were stated to occur between these two systems. This description differs from that of Zuckerkandl in the reported continuity of visible venous drainage of the bronchi from the extreme periphery to the hilum.

In regard to changes in the venous system in disease, three specimens of emphysematous lungs were described by Marchand and his co-authors,[14] in which an expansion of the bronchial veins was found. This was confirmed in two other instances of emphysema with gelatin rather than a vinylite injection medium. The connections of the bronchial with the pulmonary veins in the emphysematous lungs were described as abnormally dilated.

Ferguson, Kobilak, and Deitrick[15] studied by injection and histologic methods the lungs of a series of patients: (a) with mitral stenosis and presumably high pressure in the left atrium, (b) with congestive failure from other causes, and (c) as controls. They found the bronchial veins to be enlarged in 6 of 11 patients with mitral stenosis, and in 4 of these the changes were striking. There was some increase in the size of these vessels in some patients with congestive failure but not to the extent demonstrated in mitral stenosis.

The present work is concerned with a systematic study of the double venous systems of the lung and the effects upon them of disease.

Methods

The vinylite bronchovascular casting technique developed in this laboratory[16] (1947) was modified for the purpose of investigating the bronchopulmonary venous system. Direct injection of the veins of the bronchi from the azygos system is rendered difficult by several factors. There is first the immense venous bed with which the azygos system is connected. This makes it difficult to attain a pressure sufficient to inject small peripheral vessels before the plastic hardens within the capacious ramifying passages of the larger veins. Part of the plastic is shunted into the spinovertebral system whose communications with the azygos and retroperitoneal venous systems have been so graphically described by Batson.[17] These vessels have been demonstrated with the vinylite technique in this laboratory in experiments with dogs wherein the entire thorax was digested in acid after the introduction of vinylite into the azygos vein; the capacity of the spinovertebral system is then seen to be truly impressive.[18] Through this system the branches of both venae cavae also are injected freely.

The second difficulty results from the presence of valves at the points of inosculation of the bronchial and azygos veins. These direct the blood toward the azygos system and are usually competent in normal persons.

Despite these difficulties, the azygos vein was injected with the lungs *in situ* using vinylite at approximately 10 per cent concentration in acetone. This material was tinted yellow with luxol fast yellow G or T.* The bronchial arteries were injected from the aorta, also with the lungs *in situ*, as has been previously described.[16] This served not only to demonstrate the condition of these vessels in the specimens examined, but also to add support to the azygos cast.

The venous system was injected also from the left auricle as in our earlier method but employing more dilute vinylite for the first injection. This was accomplished through a cannula in the left auricle with the lungs inflated in a vacuum jar. The composition of the dilute vinylite is as follows: vinylite (28 per cent), 150 cc.; acetone, 150 cc.; green dye, saturated solution in acetone (filtered), 60 cc.; diatomaceous earth, 15 gm. More viscous "filled" vinylite was then introduced. In this way both fine and coarse veins, including those of the bronchi, were injected. In some instances the green vinylite could be made to penetrate into the mediastinum where it became continuous with the yellow material that had been introduced into the azygos system.

* Obtained from DuPont de Nemours Co., Wilmington, Del.

The pulmonary arteries and bronchi were injected exactly as in the original method.

In the descriptions to follow, the terminology of Jackson and Huber[19] and the designations of segmental vessels devised by Boyden[20] will be employed.

Material Surveyed

Over 140 casts were reviewed for this study. These included 65 from persons of various ages in whom there was no evidence of pulmonary disease. Almost all of these were rather coarsely injected on the venous side with viscous vinylite. Four, however, were prepared specifically as controls for the present observations, employing the method outlined above in every detail. There were 16 casts from various types of congenital heart disease, including 3 from patients with complete transposition of the great vessels and the remainder largely from patients with tetralogy of Fallot or congenital pulmonic stenosis. Two specimens were from patients with primary carcinoma of the lung and one from a lung containing metastases of carcinoma of the kidney. Twelve specimens from patients with bronchiectasis were examined. All but 2 of these were surgical specimens in which whatever injection of the bronchopulmonary veins there was, took place retrogradely from the pulmonary vessels. Eleven specimens were from patients with pulmonary tuberculosis. There were 3 patients with chronic rheumatic heart disease. Two of these had mitral stenosis, pulmonary arterial hypertension, right cardiac hypertrophy, and hemosiderosis, and the third had only slight mitral stenosis with striking insufficiency of this valve. There were 7 specimens from instances of advanced pulmonary emphysema. Another cast was from a patient with Boeck's sarcoid in an advanced state of fibrosis and one was from an instance of chronic abscess of the right upper lobe. In addition, 25 casts of the lungs of experimental dogs were studied. Most of these had had a previous ligation of the pulmonary artery, 3 were animals in whom pulmonary veins had been ligated, and 3 had had compression of the left lung by cotton mesh cloth for periods up to 4 months.

Observations

In the study of presumably normal material, variable types and degrees of intercommunication between the systemic and pulmonary venous systems were observed (the left auricle will be considered part of the pulmonary venous system in the descriptions to follow). Occasionally, large mediastinal vessels can be seen entering one or another

of the larger pulmonary veins. There is also, in some specimens, evidence of direct drainage of the wall of the heart into the left atrium (Fig. 1). It is clear that these vessels contribute desaturated blood to the pulmonary veins, just as may the bronchopulmonary veins themselves.

In regard to the veins of the bronchi, our observations are entirely in consonance with those of Zuckerkandl.[12] In the periphery, minute draining vessels are seen to originate as a plexus in the walls of the smaller bronchi and bronchioles and to drain directly into a pulmonary vein. Proximally, in the first two or three orders of subdivision of the trachea, larger venules, also in plexiform arrangement, are distributed about the bronchi. These have large T-shaped or ladder-like communications with major pulmonary veins. Some components of the plexuses are distributed distally, while others proceed proximally, whence branches are derived that enter the mediastinal veins. These are connected with pleural branches and with other veins of the mediastinum including the esophageal and pericardial. Ultimately, drainage of this entire conjoint system is into the azygos vein or its branches, the hemiazygos, accessory hemiazygos, or intercostal veins. Using the concentrations of vinylite employed, it is not possible to demonstrate vessels that connect with the azygos system draining bronchi beyond the second or third order of tracheal branches, although doubtless at least capillary connections exist between these venules and the more peripheral bronchopulmonary venous systems. This is indicated by the changes under certain circumstances of disease in which vessels are observed in an expanded state. For convenience, and in recognition of their intimate intercommunication with the pulmonary veins, all of the vessels draining the bronchi and bronchioles will hereafter be referred to as bronchopulmonary veins. This does not fully describe all of their functions, for some act as vasa vasorum while others drain the pleura.

The relation of the pulmonary veins to bronchi, especially in the large proximal bronchi, is very much like that of the bronchial arteries. They occur in net-like arrangements, that transgress the boundaries of segments and even of lobes (Fig. 2). It happens in the pleura as well as in the bronchi that branches of adjacent pulmonary veins drain the same region and are there joined by more or less sizable vessels (Fig. 3). The bronchopulmonary venules then act as intervenous shunts. It is obvious that the pulmonary veins are much less "end" vessels than are the pulmonary arteries. The latter communicate with one another only by means of vessels of capillary size.

In general, as a collateral circulation develops, it may be difficult to state whether it represents an expansion of pre-existing vessels or whether new vessels are actually formed. Under certain circumstances it is apparent that new plexuses of venules, with systemic and pulmonary connections, can develop. This has been demonstrated experimentally in this laboratory after ligation of the pulmonary veins in the dog. Here, adhesions between the chest wall and the lung in the region of the operative incision are penetrated by venules that connect the intercostal with the pulmonary veins.[18] Furthermore, in neoplastic disease, there can be found newly formed vessels presumably draining desaturated blood from this actively metabolizing tissue (Fig. 4).

Expansion of the bronchopulmonary venous system does not necessarily accompany an increase in the arterial collateral circulation. The best demonstration of this is in the experimental dog with ligated pulmonary arteries, in which an immense arterial collateral circulation develops, but there is no obvious increase in bronchopulmonary veins.[21] This is confirmed also by physiologic studies that suggest that drainage is almost entirely into the pulmonary venous system.[8] On the contrary, in the dog bronchopulmonary venous drainage leading into the azygos system is vastly increased, and in a brief period of time, after the pulmonary veins are ligated experimentally. In congenital pulmonic stenosis in man the bronchopulmonary veins may or may not be expanded and this expansion is frequently out of step with that of the bronchial arterial collateral circulation. The expansion of the venous collateral circulation is usually moderate, ordinarily representing chiefly an increase in the caliber of pre-existing vessels (Fig. 5). In certain instances, in which there is only a slight increase in collateral circulation on the arterial side, the venous side may be very significantly expanded (Figs. 6, 7, and 8).

In transposition of the great vessels the systemic and pulmonic blood streams would run independently, in parallel, were there no septal defects, patency of the ductus arteriosus, or other shunts. It might be expected that the intrapulmonic venous route (pulmonary vein-bronchial branches of the azygos) would constitute another possible shunt. In three instances of transposition in which bronchovascular casts were made, there was, however, no suggestion of enlargement of the bronchopulmonary venous bed.

To our surprise, in consideration of the observations of Ferguson, Kobilak, and Deitrick,[15] the three lungs from patients with chronic rheumatic heart disease displayed no notable increase in the venous collateral circulation. This is probably explained by technical factors,

in that the vinylite as employed may be too viscid to penetrate the finer channels. Studies of other patients may yield different results. In one specimen from a 4-year-old child with putatively congenital mitral stenosis, severe sclerosis of the pulmonary arterioles, and hypertrophy and dilatation of the right side of the heart, a slight but definite increase in the bronchopulmonary venous channels was demonstrated.

All 12 bronchovascular casts from examples of extensive bronchiectasis exhibited a moderately to markedly increased arterial collateral circulation and most showed many large anastomoses between the pulmonary and bronchial arteries. The venous collateral circulation, however, was considered to be notably enlarged in only one of these, although there was a slight to moderate increase in 8 specimens. The veins of this specimen had been injected with highly viscid vinylite "filled" with 10 per cent diatomaceous earth, as was true of most of the specimens in the bronchiectasis series. Such material will inject vessels no larger than approximately 0.2 mm. in diameter. In such instances the similarity in course and plexiform arrangement of the bronchial arterial and venous vessels is striking (Fig. 9). The venules tend to spiral less about the bronchi. A striking example of a T-shaped connection of a network of bronchial venules with a large pulmonary vein is shown in the bronchovascular cast of a bronchiectatic lobe in Figures 9 and 10. Unlike the anastomoses between the two arterial systems, these communications exist normally, but are here greatly enlarged. Furthermore, the distal end of the T is a grossly visible vessel, not only in the region of the fifth order branch of the trachea (where lies the short connecting piece of the T), but for several orders of branches beyond. The proximal limb of the T extends into the crotch between the superior and common basal bronchi of the lower lobe and thence presumably into the systemic mediastinal plexus, for that is the usual course of vessels in this position.

In the tuberculous lungs and in the example of lung abscess, the venous changes were comparable to those in bronchiectasis. There was, however, a considerable increase in the arterial collateral supply.

The most striking increase in the venous collateral system was observed in advanced pulmonary emphysema. Seven patients were studied. The clinical histories are presented in detail in the Supplement, together with descriptions of the casts in all 7 instances. In this section, therefore, the anatomical findings will be merely summarized.

In 6 specimens the emphysema was of a bullous type. Here the pulmonary veins lying in the septa among the bullae had become grotesquely elongated and thinned out, and appeared widely separated. The

attenuation of the pulmonary arteries and veins was in part associated with stretching consequent upon expansion of the bullae. A similar stretching of the bronchioles probably resulted in narrowing and therefore in further growth of the bullae by entrapment of air, thus establishing a vicious cycle. In case 7 the emphysema was finely vesicular and was not associated with right cardiac hypertrophy nor with significant clinical symptoms. Bronchopulmonary venous expansion was nevertheless notable (Fig. 24).

Since these lungs were necropsy specimens, the azygos injection was carried out as described in the section on Methods. It is noteworthy that, in contrast with the normals in which competent valves prevented injection of bronchial veins, these veins could be injected with ease in the patients with pulmonary emphysema. This incompetence of the valves is probably the consequence of the great enlargement of the bronchopulmonary veins even where they enter the azygos system.

It is apparent from inspection of the casts that the bronchial arterial circulation was expanded in all except case 7, but that in all of the specimens a much more striking increase had taken place on the venous side. The principal pulmonary venous connections of the enlarged collateral veins on the anterior aspect were with the lobar veins, particularly the inferior, and the anteromedial basal veins on the left, or the medial basal veins on the right. Posteriorly, the major pulmonary vessels were the apical veins of the lower lobes and the posterior vein of the right upper lobe (V^3 of Boyden[20]).

The expanded collateral vessels in their relations to the intrapulmonary bronchi were similar to those that have been described for the example of bronchiectasis. Their distal extensions, as grossly visible vessels from the hilum to bronchi beyond those of the fifth order branches of the trachea, are demonstrated in Figures 20, 21, and 22. A typical T vein is shown in Figures 20 and 21. The multiple communications established among these venous vessels are demonstrated best in Figures 14 and 24. The junction of the bronchopulmonary vessels with greatly enlarged pleural veins is shown in Figures 14 and 17. In general, the greatly enlarged bronchopulmonary veins from all lobes of both sides tend to meet in a plexus beneath the carina. Thence draining vessels sweep upwards, posterior to the tracheobronchial tree, to their destination in the azygos system (Figs. 11, 13, 15, 16, and 17). Thus, when the bronchopulmonary venous collateral is expanded, the pulmonary veins of adjacent segments are brought into much freer communication, and the normal shunts between pulmonary and systemic vessels are considerably enlarged.

It is of note in these patients that the venous vascular changes can take place independently of the development of cor pulmonale or overt respiratory failure, nor does the emphysema need to be of bullous type. The best evidence for this is given by case 7. In the other patients there was evidence of hypertrophy of the right side of the heart. In 5 instances the ventricles were freed of vessels, valves, and as much fat as possible and separated through the septum according to the technique employed by Snavely.[22]* This method has yielded consistent results with apparently normal hearts in the hands of different observers. In 3 of the 5 patients (Table I, cases 2, 3, and 6) the right

TABLE I

Cardiac and Ventricular Weights and Ratios

Case no.	Age	Weight of patient	Heart, original weight	Ventricles, total weight	Right ventricle, weight	Left ventricle, weight	Right ventricle: left ventricle
	yrs.	*lbs.*	*gm.*	*gm.*	*gm.*	*gm.*	*ratio*
1	62	162	450	326	115	211	0.55
2	52	91	320	223	116	107	1.09
3	52	150	375	229	121	109	1.11
5	79	180	450	297	93	204	0.45
6	58	145*	317	291	161	130	1.24

* 1½ years before death.

ventricle actually exceeded the left in weight, indubitable evidence of the cor pulmonale that was indicated also by the clinical observations. In the other 2 instances both ventricles exceeded the absolute limits of normal weight, but the ratios of the weights were within normal limits on account of hypertrophy of the left ventricle. According to Snavely, Layton, and Richar,[22] the weight of the right ventricle normally is 0.48 ± .08 that of the left. Both patients had suffered from dyspnea for many years. There was no clinical record indicating systemic hypertension in either instance. In case 1 there was polycythemia with death from cerebral infarction and hemorrhage. In case 5 there had been congestive failure with death apparently in consequence of an intramural hemorrhage in the right coronary artery. In case 4, in which the ventricles were not weighed separately, there was gross anatomical evidence of a relative hypertrophy of the right ventricle. The mechanism of death, however, seemed to be largely one of respiratory rather than cardiac failure.

* I wish to express my deep appreciation to Dr. J. G. Snavely, who dissected these specimens.

Discussion

Functional Implications of an Expanded Venous Collateral Circulation

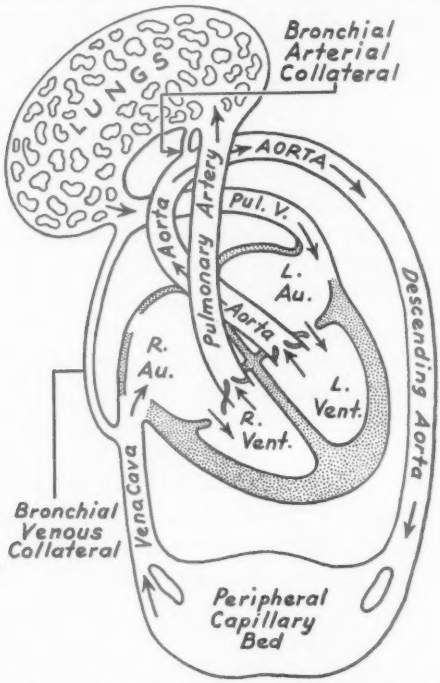
It is apparent from inspection of the casts that as the caliber of the bronchopulmonary veins increases, in view of their multiple ladder-like connections with the pulmonary veins, a by-pass of significant size is provided that could become operative in the event of occlusion of major pulmonary veins. The latter is a distinct possibility in chronic pulmonary disease, especially in the presence of recurrent infections.

Under normal circumstances it is likely that the flow in the bronchopulmonary veins is toward the right side of the heart, as would be suggested by the higher pressure in the left auricle (Opdyke *et al.*[23]). The pressure differentials, however, are small and could be reversed, were it not for competency of the valves at the bronchopulmonary-azygos junctions. It has been demonstrated in the present observations that as the bronchopulmonary collateral circulation expands with stretching of the rings at the inosculations with the azygos, these valves may indeed become incompetent. The shunt could then be from right to left, under circumstances when the systemic venous pressure exceeds the pulmonary. This may happen in cor pulmonale with failure of the right heart, for then there is no reason why the pressure in the left auricle should be elevated. Shunting of blood from right to left through these venous passages may then contribute to the desaturation and hypercapnia of the systemic arterial blood that is observed in the terminal stages of emphysema, *e.g.*, case 4 (Supplement). Furthermore, such a shunt could account for the sudden increase in desaturation observed at times upon digitalizing patients with pulmonary emphysema and cor pulmonale,[24] while there is no fall in cardiac output, nor significant increase in pulmonary arterial pressure, *e.g.*, case 3. These considerations and others require further exploration in the presence of the enlarged pathway of communication between the systemic and pulmonary venous systems (Text-fig. 2).

Mechanisms Responsible for Expansion of the Bronchopulmonary Venous Circulation

The mechanisms responsible for the expansion of the bronchopulmonary venous circulation are even more obscure than those responsible for the development of the arterial collateral system. As has been demonstrated, the two do not necessarily go hand in hand. Furthermore, the venous collateral circulation is only occasionally enlarged in notable degree merely in association with formation of granulation tissue. This may, however, be one factor.

It appears from the studies of Loeschcke,[25] confirmed in this laboratory, that the "bullae" of severe emphysema represent expanded parts of the respiratory tree more proximal than alveoli, including respiratory bronchioles and even bronchioles. Not only have the connective tissue walls of these passages become thickened, some actually having the structure of granulation tissue, but also the musculature may become notably hyperplastic and hypertrophied.[26] The venous



Text-fig. 2. In chronic pulmonary disease there must be added to the increased bronchial arterial collateral circulation which introduces the complexities of flow summarized in Text-figure 1, consideration of an expanded venous collateral circulation. This is shown here as a shunt between the pulmonary veins and vena cava. The volume and even the direction of flow are as yet incompletely known.

drainage of these structures is bronchopulmonary rather than purely pulmonary, just as their arterial supply stems from the aorta via the bronchial arterial system.

The particularly extensive collateral venous development in advanced pulmonary emphysema suggests an inquiry also into functional factors operative in this condition. One consideration is that the bronchopulmonary veins, protected as they are within the walls of the relatively rigid bronchi, would be less affected by changes in the intra-

alveolar pressure. In emphysema, as has been suggested by Macklin[27] and others, the blood pumping action, resulting from the alternating respiratory pressure changes as they affect the small vessels of a normally elastic parenchyma, is reduced. This will tend to abolish the differential negative pressure favoring the flow of blood into pulmonary venules rather than bronchial venules, that normally occurs in each inspiration. Thus, relatively more blood might be directed into bronchial venules when this pressure differential is in abeyance. For reasons not entirely clear, greater blood flow is associated with an increase in size of vessels.

Summary and Conclusions

In certain types of chronic disease of the lung, notably in emphysema, the bronchopulmonary venous circulation is greatly expanded. Since the bronchopulmonary veins communicate at many points with the pulmonary veins, they provide a by-pass in the event of occlusion of the latter. In their expanded state, the bronchopulmonary veins constitute a shunt of significant size between the right and left auricles. The direction of flow is probably from left to right until the valves at the points of junction of the bronchopulmonary and azygos veins lose their competency—as is usually true in advanced emphysema. It is then possible for the flow to be reversed. Pressure differentials on the two sides, however, are ordinarily small. Thus, the volume and even direction of flow under various circumstances remain for further investigation. The mechanisms concerned in the development of the bronchopulmonary venous collateral circulation are not well understood, but changes in the respiratory pumping action that affect especially the truly pulmonary rather than the more protected bronchopulmonary venules may be of importance, as may the development of increased interstitial tissue and muscle in the diseased lung.

For an understanding of the circulation in pulmonary disease, consideration must be given to expanded venous shunts between the right and left sides of the heart, as well as to the increased bronchial arterial circulation and to the anastomoses of the two arterial systems.

[*Supplement follows*]

SUPPLEMENT

Case Histories and Descriptions of Bronchovascular Casts of Patients with Pulmonary Emphysema

Case 1 (A–9209)

Clinical History. The patient was a male, 62 years old, with a long history of emphysema and dyspnea, admitted on August 6, 1951, in an unresponsive state.

He was first seen in the New Haven Hospital in 1934, when he had been suffering from dyspnea for 2 years. He had gained weight rapidly during the preceding 2 years. The past history is of interest, in that he had been a polisher and grinder of brass for 20 years. During the 1934 admission the blood pressure was 108/70 mm. of Hg. The chest was barrel-shaped, with poor expansion. There was resonance despite the obesity. Râles of "sticky" type were heard at both bases. There were expiratory wheezes and fremitus was diminished throughout. Roentgenographic examination of the chest suggested pulmonary fibrosis on the basis of accentuation of the bronchovascular markings. A lipiodol bronchogram revealed no evidence of bronchiectasis. At this time the red blood cell count was 5.02 millions per cmm., with a hemoglobin level of 86%. The diagnosis was chronic pulmonary fibrosis and obesity.

He was seen again in February, 1951. His complaints were of bouts of coughing productive of mucopurulent sputum for the preceding 2 years, pain in the lower portion of the back, and dyspnea. His temperature was 98.2° F.; pulse, 82; respirations, 28; and blood pressure, 140/85. He was still obese. The lungs were hyperresonant throughout. The breath sounds were bronchovesicular with scattered expiratory wheezes and coarse rhonchi. There were râles described as "medium coarse" over the lung bases posteriorly. The prostate was slightly enlarged, but rectal examination did not suggest carcinoma. There was roentgenographic evidence of a compression fracture of the tenth dorsal vertebra. He was believed to have chronic pulmonary emphysema with bronchitis and moderate fibrosis. The bone lesion was considered possibly due to metastasis from a carcinoma of the prostate.

Laboratory Data* (Blood)

Date	R.b.c.	Hgb.	W.b.c.	P.	Ly.	L.m.	E.	B
11/13/34	5.02	14	8,000	74	25	1	0	0
2/28/51	6.89	17	25,600	95	2	3	0	0
3/ 5/51	7.89	17	28,050	78	17	3	1	0

Date	NPN	Sugar	Na	CO_2	Cl	Alb.	Glob.
3/1/51	50	116	142.9	32.2	89.5	3.35	3.51

* Except as otherwise indicated, in this and subsequent tabulations of laboratory data the values for hemoglobin, albumin, and globulin are in grams per cent. The values for non-protein nitrogen and sugar are in milligrams per cent. Results of determinations of carbon dioxide, sodium, potassium, and chlorine are expressed as milliequivalents per liter. Differential white blood cell counts are given as percentages; red blood cells in millions per cubic millimeter, and white blood cells in absolute number per cubic millimeter.

There was now a notable elevation in the red blood cell count to levels between 6.89 and 7.89 millions with 17 gm. of hemoglobin. The white blood cell count was over 25,000 per cmm. with a slight shift to the left on two examinations. Material obtained by sternal puncture showed a hyperplastic cellular marrow, considered compatible with polycythemia vera. The oxygen saturation of the blood was not determined. The acid phosphatase level on March 6 was 1.6 Bodansky units. An electrocardiogram showed evidence of a moderate left axis shift.

The patient's final admission came on August 6, 1951. He was found to be coma-

tose. His temperature was 102° F.; pulse, 120; respirations, 26; and blood pressure, 110/70. The fundi showed blurred disk margins and there were several retinal hemorrhages on the nasal side of the left disk. Numerous râles were heard in the chest and there was dullness laterally and at both bases. The size of the heart again could not be determined. The abdominal reflexes were absent, but the corneal reflex was present. The Babinski sign was questionably positive on both sides. The deep tendon reflexes were hyperactive. Moist râles on both sides increased progressively. The patient became cyanotic and expired approximately 2½ hours after admission.

Clinical Diagnosis. Cerebral vascular accident. Acute pulmonary edema. Pulmonary emphysema and fibrosis. Polycythemia vera. Arteriosclerotic heart disease. Carcinoma with bony metastases, primary site undetermined.

Observations at Necropsy

At necropsy the patient was short and stocky, 156 cm. tall and 162 lbs. in weight. There was a striking degree of arteriosclerosis and arteriolosclerosis. The heart was slightly enlarged, weighing 450 gm. The right and left ventricles, particularly the latter, appeared somewhat thickened (Table I). The lungs were emphysematous. The emphysema was of a fine bullous type. Microscopically, there was pulmonary edema and focal anthracosis without nodular silicotic lesions. There was a zone of hemorrhage in the right parietal lobe of the brain. This apparently originated in an infarct and extended to the right lateral ventricle. Extramedullary hemopoiesis, especially erythroid, was demonstrated in the spleen and liver, with enlargement of these organs. The bone marrow was hyperplastic. A minute carcinoma was found in the prostate. There was a healed compression fracture of the tenth dorsal vertebra.

Description of Cast

Left Lung. The larger bronchopulmonary veins on the left were derived from branches of the inferior vein. There was a large venous plexus that stemmed from the anterior aspect of an anteromedial segmental vein. One branch of this plexus proceeded downward along branches of the medial sub-segment, and the other swept proximally toward the hilum behind the main pulmonary veins. A second series of large bronchopulmonary veins stemmed from the very base of the superior segmental vein of the lower lobe (V^6 of Boyden,[20] Fig. 11). One set, arising inferiorly, was a very large intrapleural and mediastinal plexus, injected in green from the pulmonary veins that communicated with a similar intricate plexus of vessels, some of which were esophageal and were injected in yellow from the azygos system. The other set extended upward behind the left hilar structures.

Right Lung. On the right side, the largest bronchopulmonary vein was derived from the middle lobe vein on its anteromedial aspect. This vessel was recurved upon itself, yielding many branches to the region of the mediastinal and interlobar pleura and then sweeping proximally to the common basal and common middle lobe bronchi. Additional sizable collateral veins were derived from the posterior segmental vein of the upper lobe (V^3 of Boyden[20]). These swept upward on the medial aspect of the intermediate bronchus. V^6 also gave rise to a vessel almost parallel in its course, but embracing the medial and posterior aspects of the intermediate bronchus.

Throughout this specimen there were innumerable fine bronchopulmonary veins which were derived from peripheral branches of pulmonary venules. They were best seen on the medial aspect of the medial segment of the right middle lobe where they could be traced proximally and were seen to be in communication with the bronchopulmonary veins derived from a major pulmonary vein that drained this segment of the bronchial tree. Small pleural veins were found to be in direct communication with the branches of pulmonary venules on the medial aspect of the left lower lobe. The pulmonary veins were remarkable for their long, tortuous, and recurved course. The bronchial arteries in this specimen were considerably enlarged.

Case 2 (A–9039)

The patient, a railroad conductor, 52 years of age, was admitted on March 12, 1951, with the chief complaint of difficulty in breathing of 8 years' duration. This symptom had had an insidious onset and was associated with a cough productive of approximately 1 cupful of yellow sputum daily. The sputum had been streaked with blood only during the 3 weeks preceding admission. During that time dyspnea had become accentuated. Three days prior to admission he passed a "tarry" stool and there had been several since. Edema of the ankles was noted several months earlier.

Physical Examination. Temperature, 99° F.; pulse, 98; respirations, 34; blood pressure, 73/44 to 130/70 mm. of Hg. The heart sounds were faint and there were no murmurs. There was no evidence of cardiac enlargement upon physical examination. The chest was hyperresonant and the breath sounds were distant throughout. There were "medium" râles at both bases. The diaphragm seemed to be very low. The skin was a mottled red-purple and was cold.

Course in Hospital. Oxygen was administered immediately by mask, following which the blood pressure rose to 110/60, and later to 130/70. A striking leukocytosis was found although the temperature was subnormal. The pulse, however, was increased to about 120. A blood transfusion and intravenous infusion of glucose and saline solution were given also in the attempt to raise the blood pressure. He was given 500 cc. of 2% sodium chloride to elevate the low sodium level. It seemed necessary to supply oxygen only 10 minutes of each half hour by mask. It was then decided to supply oxygen by nasal catheter, but before the catheter had been placed, the patient expired on the day after admission. The urine was not examined.

Laboratory Data (Blood)

Date	R.b.c.	Hgb.	W.b.c.	P.	Ly.	L.m.	E.	B.
3/12/51	2.80	10	44,000	98	2	0	0	0

Date	NPN	CO_2	Cl	Na	Alb.	Glob.	K
3/13/51	73	36.2	73	124.0	2.32	3.13	4.8

Observations at Necropsy

At necropsy the heart weighed 320 gm. Both right auricle and ventricle were hypertrophied and strikingly dilated, as were the pulmonary conus and pulmonary artery. The left ventricle was not increased in thickness or weight (Table I). There were numerous old pleural adhesions, and numerous blebs and bullae were found throughout both lungs. Emphysema was diffuse and was most striking in the left apex which was almost entirely replaced by bullae. Many fine and coarse nodules could be palpated in the very soft and spongy pulmonary substance. Microscopically, there was evidence of focal pneumonitis in the left lung. Other alveoli contained hemosiderin-filled phagocytes and their walls were extremely thin.

Description of Cast

The main sources of collateral venous supply were as follows:

Left Lung. Upper lobe: A vein to the upper part of the left lung (V^1 of Boyden[20]) was the source of a very large trunk on the anterior aspect that looped over a bronchus to pass posteriorly. There were also several smaller bronchopulmonary branches derived from the lingular veins. Another very large branch, that appeared to be rather peripheral in origin, was seen on the anterior aspect of the anterior bronchus roughly parallel to the first branch of V^1. Lower lobe: There was a plexus of moderate size derived from the principal vein of the anteromedial segment ($V^{7\text{-}8}$). On the posterior aspect, the superior segmental vein (V^6) was the source of a large bronchopulmonary plexus. This connected with plexuses that led to a large rectilinear channel that ended in branches of the hemi-azygos and accessory hemi-azygos veins.

Right Lung. The largest bronchopulmonary stem of the right lung came from the inferior vein (Fig. 12); one branch swung anteriorly in relation to the middle lobe bronchus; another joined a large subcarinal plexus. The latter then entered the azygos vein (Figs. 12 and 13). Another branch embraced the intermediate and upper lobe bronchi in a network of vessels. A second major set of vessels was derived from the posterior segmental vein (V^3). This plexus lay between the lateral aspect of the intermediate bronchus and the medial aspect of the posterior bronchus. Smaller plexuses were derived from the anterior segmental vein. There were also small pleural branches stemming from V^6.

This cast represents a very coarse injection; consequently its component injected plexuses must be of very large caliber. There were numerous huge cavities each supplied by several adjacent bronchi, clear evidence of the conjoint nature of some of the bullae. When the sacs were not filled by plastic, a rete of the vessels remained, whose distribution conformed to the shape of the sac whose outlines were not otherwise delineated. The vessels in the walls of these sacs were greatly elongated and thinned out, as were the bronchi that were also displaced and subject to stretch with the expansion of the bullae. There was moderate enlargement of bronchial arteries.

Case 3 (A–9136)

The patient was a Negro "junk man," 52 years old, who had been admitted to the New Haven Hospital seven times—each time with a chief complaint of dyspnea. He was first seen in November, 1950, when, in addition to dyspnea, he complained of anorexia and of loss in weight of 30 lbs. These difficulties had begun approximately 1 year before his admission. A radiographic examination of the chest, 8 months before admission, showed nothing remarkable, but a film made 5 months later showed enlargement of the hilar lymph nodes and a diffuse infiltration involving both lung fields. There was also evidence of slight cardiac enlargement.

Past History. A diagnosis of syphilis was made in 1931. The patient received potassium iodide and bismuth therapy between 1938 and 1940 and an intensive course of penicillin in 1948. The blood was still serologically positive in 1940, but was negative at more recent visits. There was also a suggestion of duodenal ulcer with active bleeding on several occasions.

In his work in handling trash, the patient was reported to have been exposed to broken fluorescent tubes. He sustained a cut and a specimen was taken for biopsy of the swollen region of the cut and of a lymph node on November 28, 1950, after the skin had healed. The skin showed a granulomatous process resembling the lesion of Boeck's sarcoid which was considered compatible with berylliosis. Biopsy of the liver demonstrated a granulomatous lesion resembling that of the skin. During a subsequent hospital admission, serum globulin was found to exceed the albumin, the values being 4.75 and 3.12 gm. per 100 cc., respectively. He was given ACTH followed by cortisone late in 1950 and early in 1951 with subjective improvement, but there was no apparent change when the chest was repeatedly examined radiographically.

During his fifth admission in April, 1951, pulmonary function studies * immediately before and after digitalization gave the following results:

	Pre-digitalis	Post-digitalis
Vital capacity	1481 cc.	1430 cc.
Maximum breathing capacity	49 l.	60.9 l.
Arterial O_2 saturation	92%	86%
After breathing O_2 rose to	100%	96%
Systemic blood flow	3.76 l./min.	4.3 l./min
Pulmonary arterial pressure	27/13 mm. Hg	37/16
Right ventricular pressure	33/5 mm. Hg	43/2

* By Dr. Frank D. Gray, Department of Internal Medicine.

In April, 1951, the roentgen interpretation was as follows: "diffuse reticular stippling throughout both lung fields compatible with sarcoid or berylliosis."

Last Admission. The patient's last admission was on June 3, 1951. His dyspnea had increased notably and was associated with several mild chills, fever, and general malaise. Upon physical examination, he appeared to be acutely ill and was markedly dyspneic. Blood pressure was 125/80 mm. of Hg; pulse, 120; temperature, 104.5° F. The chest showed practically no respiratory excursion and breathing was abdominal. The point of maximal cardiac impulse was in the anterior axillary line. There was sinus tachycardia. The sounds were of good quality and the pulmonic second sound was loud and ringing. No murmurs were heard. The lungs were clear to percussion. Numerous moist râles were audible at the right base, extending into the right axilla and râles in smaller numbers were heard also at the left base. Scattered harsh rhonchi were audible throughout. The firm non-tender edge of the liver was 2 to 3 finger-breadths below the costal margin. There was no edema of the lower extremities. Blood culture was negative. Roentgenograms of the chest showed extensive bilateral infiltrations compatible with Boeck's sarcoid or berylliosis. In addition, there was coalescence of infiltration in the left upper lobe suggesting superimposed infection. The heart also appeared large.

He continued to cough almost constantly and the cough was productive of small amounts of white mucoid sputum. His fever continued to rise, reaching 105° F. despite the administration of large doses of penicillin and terramycin. The patient became increasingly short of breath and died on the fifth day after admission.

Laboratory Data (Blood)

Date	NPN	Sugar	Na	K	CO_2	Cl	Alb.	Glob
4/4/51	29	76	132.5		24.4	100	2.36	4.10
6/4/51	25	101	131.3	5.0	24	97.5		

Date	R.b.c.	Hgb.	W.b.c.	P.	Ly.	L.m.	E.	B.
6/3/51	3.74	13	6,550	82	16	2	0	0

The final clinical diagnosis was berylliosis; and syphilis and duodenal ulcer based on the history.

Findings at Necropsy

The heart weighed 375 gm. There was evidence of moderate hypertrophy and dilatation of the right ventricle, the wall of which was 5 mm. thick. There was no notable change in the left ventricle (Table I). The azygos vein and the aorta with its bronchial arterial branches were injected *in situ*. Following this procedure, the left lung was dissected off, cultured, and examined microscopically. The broncho-vascular cast of the right lung, aorta, and azygos venous system was then completed. Spectographic analysis of the left lung by Robert G. Keenan, Sanitarian, Environmental Investigations Branch of the United States Public Health Service in Cincinnati, Ohio, showed the lungs to be negative for beryllium, with a content of less than 0.005 μg. of this material. Grossly, the lungs appeared almost completely airless except for emphysematous blebs seen especially in the upper parts of the lobes. The lungs were generally firm, but no gross nodules were found. Microscopic examination of the left lung revealed large, irregular zones of fibrosis. There were many groups of epithelioid cells, lymphocytes, and mononuclear elements forming small granulomata. Occasional mononucleated giant cells, some containing large vacuoles, were found also. For the most part, this response was in the form of large, irregular masses and not as discrete granulomata. Microscopic examination of the liver showed marked passive congestion. There were a few small zones of necrosis and fibrosis, but

no definite granulomata were seen, such as were observed at biopsy. There was no generalized enlargement of the lymph nodes, nor were granulomas found in any of the other viscera. Neither a duodenal ulcer nor signs of syphilis were found.

Description of Cast

Only the right lung was available, the left lung having been sectioned. The most striking feature was an immense subcarinal venous plexus. The largest source of the veins entering this plexus was the superior vein, opposite the region of entrance of the middle lobe veins (Figs. 14 and 17). This vessel swung medially anterior to the pulmonary artery and gave rise to several main branches joining a pleural plexus, and joining the large subcarinal plexus.

The pleural plexus will be described first. This was a complex of twisted bronchopulmonary veins that had their origin, in part, from the lower lobe where they were seen to communicate with the peripheral ends of some of the medially placed intrapulmonary venules. This pleural plexus was joined inferiorly to the subcarinal group of vessels (Fig. 17). Superiorly, it drained into the azygos vein at a point represented by the uppermost arrow (no. 1) in Figure 17. The subcarinal plexus extended posteriorly and was drained by two additional large branches into the azygos vein (opposite arrows nos. 2 and 3 in Fig. 17, and shown also in Figs. 15 and 16).

An additional plexus in communication with the subcarinal plexus was an interlobar group of vessels on the posterior aspect of the hilum. Its branches extended more or less longitudinally in a network along all of the major bronchi. Some elements of this plexus were comitant with the bronchial arteries.

A clear-cut connection was shown between a small pulmonary venous branch, lying inferiorly and anteriorly to the anterior division of the anterior bronchus, and the plexus that joined branches of the huge bronchopulmonary vein that was derived from the superior vein. The bronchial arteries were slightly enlarged.

Case 4 *

The patient was a 56-year-old man who was admitted to Newington Veterans' Hospital on January 16, 1951, with the chief complaint of shortness of breath of 3 years' duration. The diagnosis of emphysema had been made by his personal physician and confirmed at the Massachusetts General Hospital. Treatment was with oxygen and a sympathomimetic aerosol. His condition did not change appreciably until July, 1950, when dyspnea increased. He was studied thoroughly again at the Mt. Auburn Hospital in Cambridge, Massachusetts, during December, 1950. There his arterial oxygen saturation was found to be 90 % and the CO_2 content of arterial blood was elevated, at 37.4 m.eq./liter. There was roentgenographic evidence of marked emphysema. Treatment was again with oxygen and aerosol and, in addition, digitalis, aminophyllin, and a course of ACTH.

Past History. The patient had been an engineer in the soft coal mines of Pennsylvania from 1920 to 1923. There was no exposure to dust. His occupation since that time had consisted largely of office work in various industrial plants. For the past 10 years he had noticed a "wheezing" in his chest on occasion, especially at night. There was a history of pneumonia at 2 years and of the usual childhood diseases.

Physical Examination. Upon admission, the blood pressure was 150/90 mm. of Hg; pulse, 90; temperature, 98.6° F. The patient appeared to be slightly cyanotic and dyspneic even while sitting in bed. The anteroposterior diameter of the chest was increased. There was striking resonance with diminution of fremitus. The breath

* Permission to present clinical data on this case was granted by the staff of the Newington Veterans' Administration Hospital.

sounds were distant, with a short inspiratory phase. A few fine, transient râles were heard at both bases posteriorly. The heart sounds were distant, without murmurs. The left border of the heart as outlined by percussion was just to the left of the nipple line. Cardiac dullness extended 2 cm. to the right of the midline in the fourth interspace. There was no evidence of peripheral edema.

Clinical Course. During this admission the venous pressure was found to be elevated to 220 mm. of water. The circulation time was 27 seconds (Solu-B method[28]). At admission the blood CO_2 was elevated at 49.5 m.eq./liter (80 vol. %). By fluoroscopy the diaphragm was seen to be low and flat. There was extreme radiolucency of the lungs.

Pulmonary function tests were performed on February 12, 1951. The reserve air both supine and standing was 614 cc. The complemental air was 1030 cc. and vital capacity standing was 1290 cc. The residual air was 5991 cc. and the total capacity 6461 cc. (considered 113% of normal). The residual air was 80% of total capacity, normal being considered 31%. The maximum breathing capacity was 13.7 liters per minute. The percentage of breathing reserve to maximum breathing capacity was 60. At rest the arterial oxygen content was 14.5 vol. % with an oxygen capacity of 18.2 vol. % (81% saturation). After exercise the saturation fell to 76%.

Beginning on February 17, the patient had an elevation of temperature to 101.8° F. and symptoms suggestive of influenza which was then epidemic. There was no apparent change in the roentgenogram of the chest at this time. Beginning on February 22, the patient became disoriented and paranoid. There were episodes of intense cyanosis. On February 24 the CO_2 was found to be 55 m.eq./liter (122 vol. %). The pH of the blood at this time was 7.25. A liter of 1/6 normal lactate solution was given, without apparent change in the chemical composition of the blood or in the mental state. Pneumoperitoneum was initiated on February 26 when 1000 cc. of air were introduced, and this procedure was repeated weekly. Again there was no apparent change in the patient's condition. Aerosol bronchodilator drugs were administered as well as penicillin. The former seemed to produce palpitation and consequently were discontinued. On March 19, 1951, the patient fell out of bed and fractured the right femoral neck. No operative procedure was performed.

It was believed that improvement might follow reduction of the metabolic rate and an attempt was made to accomplish this by the administration of propylthiouracil, 100 mg. three times daily. On April 17, 1951, serum sodium was 159 m.eq. From mid-February to the patient's death, his condition remained the same. Late in April the CO_2 varied between 44 m.eq. (100 vol. %) and 60 m.eq. (130 vol. %), with chlorides between 81 and 95 m.eq./liter. Hemoglobin was relatively low throughout this time. On April 24 the patient became intensely cyanotic and there was evidence of pulmonary edema. Similar episodes occurred until his death on April 27, 1951.

Significant Laboratory Data on the Blood

Date	Hgb.	W.b.c.	P.	Ly.	L.m.	E.	B
12/—/50	13.6						
1/16/51	12.5	9500	51	44	1	0	1
4/—/51	10.5						

Date	NPN	CO_2	Cl	Na	K	Blood pH	O_2 Saturation %
12/—/50	29	49.5	90				90
1/16/51	28	36.4	95				
3/ 6/51		49	83				
4/ 5/51	35	60					
4/17/51				159	4.1		
4/27/51		58	87				
12/24/51	33	55	81			7.25	

The final clinical diagnosis was chronic severe pulmonary emphysema. It was believed that the patient's demise was associated largely with extreme emphysema, and with respiratory rather than cardiac failure.

By the usual routine tests, the urine was not remarkable.

Observations at Necropsy

At necropsy the heart weighed 420 gm. There was hypertrophy of the right ventricle, the thickness of the non-trabeculated portion of the wall being 6 mm. The left ventricle did not seem hypertrophied, but each chamber was not weighed separately. There was moderate sclerosis of the coronary arteries, without complete occlusion. Microscopically, there were foci of fibrosis and focal necrosis of small groups of myocardial fibers in the wall of the left ventricle. Grossly there was bullous and more finely vesicular emphysema. The former was striking in the apical regions. Histologically, there was emphysema and interstitial fibrosis.

Description of Cast

Left Lung. On the anterior aspect of the left lower lobe the antero-medial segmental vein was the source of a plexus that swung beneath the carina and between the upper and lower lobe bronchi (Fig. 18). On the posterior aspect the stoutest branch was derived from the superior segmental venous branch of the inferior vein (Fig. 19). This pursued a course upward, crossed the upper lobe bronchus posteriorly and then proceeded parallel with the stem bronchus of the left lung on its lateral aspect into a very tortuous vessel destined presumably for the accessory hemi-azygos. Small branches were sent downward into the hilar tissues posteriorly to the inferior vein.

Right Lung. In the right lung the largest plexus stemmed from the superior vein of the lower lobe (V^6) and also from an interlobar branch of the posterior vein of the upper lobe (V^3).

Case 5 (A–9042)

Clinical History. The patient was a 79-year-old white man who was admitted to the New Haven Hospital on March 14, 1951, complaining of severe shortness of breath of 2 days' duration. For 5 or 6 years there had been some dyspnea upon exertion or when recumbent and there had been wheezing for "many years." Two days before admission there was a sudden increase in shortness of breath, associated with productive cough and a fever to 101° F. The patient had been digitalized 3 years previously. Edema of the ankles at night had been noted for 5 or 6 months. Orthopnea was severe enough to require three pillows at night.

Physical Examination. Upon admission the temperature was 101° F.; pulse, 120; respirations, 36; blood pressure, 116/86 mm. of Hg. The patient was a large, well developed male who did not appear chronically ill, but was breathing deeply and rapidly with an oxygen mask. There was an increase in the anterior-posterior diameter of the chest, which was resonant throughout. The breath sounds were diminished. A few musical râles were heard in both lungs and these became coarse following cough. The size of the heart could not be determined accurately, but it was believed that percussion dullness extended to the anterior axillary line. The heart sounds were faint. No murmurs were heard and the rhythm was regular. There was a moderate pitting edema of the feet and lower legs.

Course in Hospital. On the day after admission the chest appeared to have cleared rapidly following the administration of aminophyllin. Without oxygen, there was slight cyanosis of the nailbeds, but the patient appeared to be dyspneic. Later in the day cyanosis increased and persisted, despite oxygen, and the breathing became stertorous. An "asthmatic" episode occurred, with dyspnea and cyanosis. Electrocardiographic examination on the day after admission was suggestive of a posterior

myocardial infarct. The blood pressure fell to approximately 95/70 mm. Hg. There was no relief following the administration of epinephrine. Late in the afternoon the patient suddenly expired.

Laboratory Data (Blood)

Date	R.b.c.	Hgb.	W.b.c.	P.	Ly.	L.m.	E.	B
3/14/51	5.00	14	14,300	88	7	4	0	1

Date	NPN	Sugar	CO_2	Cl	Na
3/14/51	60	131	26.2	101	142.4

Urine

Date	pH	Sp. gr.	Alb.	Acetone	W.b.c.	R.b.c.	Sediment
3/14/51	5.5	1030	3+	0	occ.	occ.	occ. granular cast in catheterized specimen

Final Clinical Diagnosis. Pulmonary emphysema; arteriosclerotic heart disease with cardiac enlargement.

Observations at Necropsy

There was generalized arterio- and arteriolosclerosis at necropsy. The heart weighed 450 gm. There was a healed infarct of the posterior wall of the left ventricle. Both ventricles were hypertrophied and there was dilatation of the right side. There was recent hemorrhage in the wall of the right coronary artery with marked narrowing of the vessel, but without total occlusion. This was interpreted as being probably responsible for death. The lungs were diffusely emphysematous and adherent to the chest wall by numerous fibrous adhesions. A few firm nodules could be felt within the pulmonary substance. Emphysema was of grossly bullous and in part of finely vesicular types. Microscopically, there was evidence of pulmonary congestion, edema, and of marked emphysema, without acute pneumonitis.

Description of Cast

Left Lung. In the left lung there was a dense interlobar plexus whose principal feeders were: (1) The inferior lingular vein. This entered a large network, some branches of which communicated with others in a plexiform arrangement along the common basal bronchus. At least one of these branches was derived by a complex route from a proximally crossing branch of the medial basal vein of the lower lobe. Some of these vessels were seen to accompany branches of the bronchial artery. (2) In the upper lobe the largest identifiable bronchopulmonary venous stem was derived from the upper lobe vein that represents the common drainage of the anterior and posterior segments (V^1 of Boyden[20]). Numerous supplementary bronchopulmonary venules were seen here. It was apparent that some parts of the hilar plexus extended for a considerable distance along the segmental bronchi.

Right Lung. The major sources of the venous collateral system in the right lung were as follows: (1) Principally, the posterior segmental vein (V^3) in its interlobar course, which extended a large bronchopulmonary vein upward into the upper lobe and another downward on the posterior aspect of the intermediate bronchus. (2) The medial basal vein was likewise the source of a major network chiefly applied to the anterior segmental bronchi. A remarkable T vein was demonstrated along a medial branch of the right middle lobe bronchus (B^5). This could be traced to a tertiary branch of this bronchus (Figs. 20 and 21). There was a magnificent demonstration within the left upper lobe of innumerable tortuous vessels derived from peripheral pulmonary venules that became so closely applied in plexiform fashion to the bronchi as to remove all doubt concerning their identity as bronchopulmonary vessels. Connections with pleural venous plexuses were well shown on the medial aspect of the left lower lobe in the region of the anteromedial segments. The bronchial arteries were enlarged to a moderate degree.

Case 6 (W[1])*

The patient had been admitted to the Waterbury Hospital about twenty times and once to the New Haven Hospital during an interval of 4 years, with the chief complaint of dyspnea and, later, of swelling of the extremities. He was first admitted to the Waterbury Hospital on June 18, 1948, with a history of increasing dyspnea upon exertion for the previous year. This had been slowly progressive, with periodic exacerbations. For 4 or 5 days prior to admission, the dyspnea had become so severe that he had been unable to work. He had had a chronic cough productive of green-tinged sputum that was never sanguineous. In 1948 he was 55 years of age. He had worked in a brass mill for 7 years. Beryllium had been in use in the same shops but the patient had had no direct contact with it. There was nothing of note in the family history or past history.

Upon physical examination at the first admission he appeared well nourished. There was minimal cyanosis of the lips. The anteroposterior diameter of the thorax was increased and there was some sternal bulging. Wheezing and whistling sounds and high pitched râles were heard throughout both lung fields. The blood pressure was 120/80 mm. of Hg. The heart was enlarged. There was striking clubbing of the fingers and the nail beds appeared dusky. An electrocardiogram using the standard limb lead and $CF_{2,4, \text{ and } 6}$ showed a right ventricular preponderance and an electric axis of + 120°. Roentgenographic examination of the chest demonstrated slightly prominent bronchovascular marking but there was nothing suggestive of pneumoconiosis. The red blood count was 5.3 millions with 102% hemoglobin. There was 1 plus albumin in the urine.

The patient remained improved during the next few months on ammonium chloride, but a sudden increase of severe dyspnea and productive cough led to readmission on December 8, 1948. The most notable finding was a red blood cell count of 7.04 millions with 138% hemoglobin. Otherwise his condition seemed little altered. The patient improved rapidly on bed rest after digitalization. On several subsequent admissions for similar complaints the findings did not deviate significantly. Bronchography in May, 1949, revealed no evidence of bronchiectasis. The total proteins were then 6.7 gm. %, with albumin 4.2 gm. He was maintained on digitalis and diuretics.

The patient was admitted to the New Haven Hospital on July 11, 1950. The blood pressure was 118/58; pulse, 130; respirations, 28. The skin was cyanotic. There was pitting edema of the lower extremities to the level of the perineum. The thorax was hyperresonant except for dullness posteriorly at the bases and apices where coarse räles were heard. The liver was palpably enlarged with its border 8 cm. below the costal margin. The red blood cell count was now 5.62 with hemoglobin of 14.5 gm. The urinalysis revealed nothing of note. On July 12, 1950, the non-protein nitrogen was 40 mg. %; CO_2, 38.2 m.eq./liter; chlorides, 87 m.eq./liter; sodium, 140.5. Pulmonary function tests (Dr. M. H. Williams) were performed on this day with the following results: maximum breathing capacity, 23.7 liters/min.; tidal volume, 732 cc.; minute volume, 14.7 liters/min.; vital capacity, 1596 cc.; breathing reserve, 8.44 liters. The spirogram strongly suggested pulmonary emphysema.

On July 14, cardiac catheterization was performed. The right ventricular pressure was found to be 51/−4 mm. of Hg, and the pulmonary artery pressure was 51/20 mm. of Hg. The right auricular pressure was approximately 0. A blood sample from the right auricle contained 71.4 vol. % of oxygen (39.7% saturation). The arterial blood was 60.6% saturated, contained 11.2 vol. % of O_2, which rose to 17.4 vol. % (94.1% saturation) after breathing 100% oxygen for 5 minutes. Failure to obtain full saturation at this point suggested some degree of right to left shunt, presumably within the lungs. The patient was discharged under digitalis on July 15, 1950. During

* Permission to use data from this patient was granted by Drs. O. J. Bizzozero and C. Wilson of the Waterbury Hospital.

subsequent admissions edema of the ankles and dyspnea were found to have increased. There was also evidence of ascites. Paracenteses were performed and mercurial diuretics administered. Electrocardiographic examination again showed a right axis deviation. Erythrocyte count again was found to be over 7 millions and 650 cc. of blood was withdrawn with some relief.

Final admission was on November 4, 1951, at the Waterbury Hospital, after the patient had been in coma for 36 hours. When seen he had regained consciousness. He was dehydrated and there was notable cyanosis. The blood pressure was 80/50 mm. of Hg, the chest was tympanitic, and coarse râles were heard at both bases. The heart sounds were rapid and distant. Several paracenteses were performed and one of the small abdominal wounds was found to contain purulent material. The hemoglobin was 16.5 gm. with a red blood cell count of 5.6 millions; the white blood cells were 12,800 with 95% neutrophils. The non-protein nitrogen was 62 mg. %, with CO_2 of 25 m.eq./liter, and chlorides of 90 m.eq. The patient complained of abdominal pain, particularly in the upper quadrants. There was abdominal distention and paracentesis yielded almost 5 liters of straw-colored fluid. The patient expired on the 15th hospital day, after increasing lethargy.

Findings at Necropsy

At necropsy, in addition to striking pulmonary emphysema, largely of finely vesicular, but also focally of bullous, type, there was peritonitis apparently associated with repeated paracenteses. The heart was only slightly increased in weight, but the right ventricle was hypertrophied (Table I). The trunk of the pulmonary artery was large and thick-walled. Microscopically, the pulmonary changes were those of emphysema. There were small scars believed to represent organized focal pneumonitis. Many of these were subpleural. There was no evidence of a specific pneumoconiosis, nor were the blood vessels notably sclerotic.

Description of Cast

There was considerable enlargement of the bronchial arteries. A large bronchial branch in this instance was derived from the right subclavian. The bronchopulmonary veins on the left originated as major branches of the following: The lingular vein gave rise to a subpleural plexus that communicated with a similar plexus taking origin from the posterior aspect of the inferior vein of this side. Additional plexuses came from the medial aspect of V^{7-8}. The superior segmental vein of the left lower lobe was the source of vessels that formed a posterior plexus, chiefly on the medial aspect of both the lower and upper lobes. On the right side the larger bronchopulmonary venous systems stemmed from V^{3} on the medial aspect of its interlobar course. The major trunks derived from this vessel proceeded largely upward on the posterior aspect of the intermediate and stem bronchi. Additional plexuses were derived from the inferior vein. They were distributed chiefly on the medial, anterior, and lateral aspects of the intermediate and lower lobe bronchi.

Throughout these lungs many of the bronchi, particularly in the distribution of the superior bronchus of the left lower lobe, were notable for the intimate bronchopulmonary venous plexuses that stemmed from peripheral venules. Many of these tiny branches proceeded proximally, ultimately to become tributaries of the azygos veins (Fig. 22). Minute pulmonary venules also contributed to fine plexuses of tortuous pleural venules (Fig. 23).

Case 7 (A–9346)

The patient was a man, 68 years of age, who had been shot through the head. He had been active and had had no physical complaints. At necropsy pulmonary emphysema was noted. The lungs were large and almost met across the midline. The emphysema consisted of a diffuse fine expansion of the terminal air spaces, without large bullae. The heart weighed 340 gm. and showed no evidences of hypertrophy or dilatation of the right side.

Description of Cast

Left Lung. The largest bronchopulmonary branch was derived from an anomalous inferior lingular vein (VX^5) that drained into the inferior vein. There were communicating branches from this vessel to a very extensive subcarinal plexus and also to a pleural plexus. A second series of bronchopulmonary veins of this side was derived from V^{7-8}, and a third series was derived from V^8 itself (Fig. 24).

Right Lung. An upper lobe plexus stemmed from V^3. These bronchopulmonary veins embraced the intermediate bronchus on both the anterior and posterior aspects, particularly the latter. The posterior branches of this plexus communicated with the subcarinal plexus that has been described. A very extensive set of collateral veins came from the right inferior vein. This gave off some branches that swept upward to the subcarinal plexus; others proceeded medially to the region of the middle lobe and there were many that swung downward along the branches of the lower lobe bronchi. The subcarinal plexus thus represented the junction of all elements of the venous collateral circulation of both sides (Fig. 24). The clearest demonstration of the long course of a collateral vessel was presented along branches extending from the anterior vein of the right side. These minute venules could be traced along branches of the anterior bronchus, representing sixth order subdivisions of the trachea. Here they met venules that extended proximally from distal pulmonary venules of adjacent segments.

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LEGENDS FOR FIGURES

FIG. 1. Posterior view of a vinylite bronchovascular cast showing a large mediastinal vein entering the superior vein of the left lung. The tortuous vessel is visible in its descent beneath the carina. A large intramural vein enters the posterior surface of the left atrium directly. The descending aorta, seen as a black mass at the left, has been transected to expose these vessels.

FIG. 2. Cast showing a large bronchopulmonary vein originating from the anteromedial segmental pulmonary vein and proceeding proximally. The intimate relation of the bronchopulmonary vessel to the bronchus is similar to that of the bronchial artery as is its plexiform structure. A vascular loop is clearly seen. The lobar boundary is transgressed and a complex of the vessels arches beneath the upper lobe bronchus to be distributed along its branches. The dark vessel above the upper lobe bronchus is the pulmonary artery.

FIG. 3. Two pulmonary veins in the lingular segment are joined by a large tortuous complex of vessels within the pleura, at the right, that probably represent bronchial venules common to both. This type of cross connection among adjacent venules is common within the substance of the lung in the walls of the bronchi. Cast of the left lung of a patient with tetralogy of Fallot.

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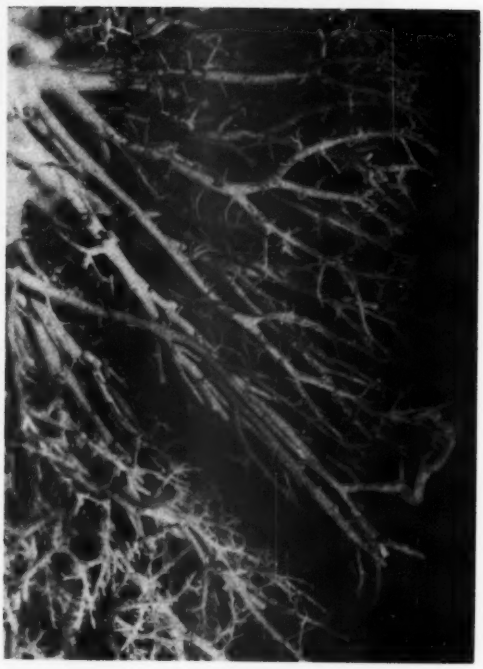

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FIG. 4. The vessels within the substance of a carcinoma of the lung have been injected. There is a very complex fan-shaped mass of intercommunicating venules that enters a small pulmonary vein. These are considered to be newly formed within the substance of the tumor since structures of this type are not found normally.

FIG. 5. A venous ring is clearly seen in a bronchovascular cast from a patient with pulmonic stenosis. A branch of the superior and a branch of the inferior vein of the right lung are seen to be joined by somewhat tortuous, narrow vessels on the medial hilar aspect. These are considered to be bronchopulmonary veins. From the medial side of the junction of the latter there originates a stem of approximately the same size extending upward. This vessel is destined for the mediastinal plexus of systemic veins for ultimate drainage into the azygos system. Enlarged bronchial arteries (black) are seen crossing the bronchi posteriorly.

FIG. 6. Anterior view of bronchopulmonary cast from a patient with tetralogy of Fallot showing a large mass of bronchopulmonary veins beneath the carina derived from the right superior vein. Anterior to the trachea is another plexus that joins the subcarinal plexus, but derived from bronchopulmonary venules that loop over the left main bronchus. These connect the anterior plexus with networks of bronchopulmonary vessels on the posterior aspect of the lung (Fig. 7).

FIG. 7. Posterior view of the cast shown in Figure 6. Tortuous bronchopulmonary veins are visible above the left main bronchus as a twisted mass of irregular vessels, one of which is seen to loop over the left main bronchus. Other branches pass beneath the carina to join the subcarinal plexus shown in Figure 6. All of these vessels are derived from various pulmonary veins, chiefly the superior segmental vein of the lower lobe. From the subcarinal plexus branches proceed downward to join a transverse branch that connects the azygos and hemi-azygos veins.

FIG. 8. It is apparent that the bronchopulmonary veins derived from veins of both sides enter a common plexus that ultimately drains into the azygos venous system. This drawing represents a composite view of the distribution of veins shown in Figures 6 and 7. In this instance the aorta arches over the right main bronchus. The azygos and hemi-azygos veins appear black.

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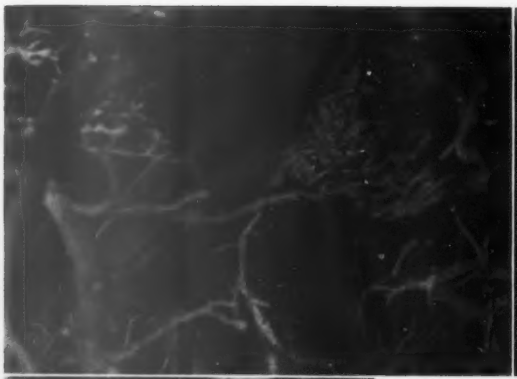

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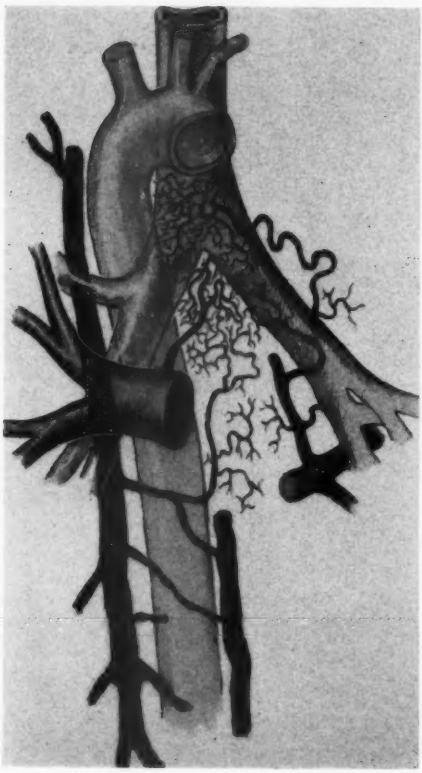

FIG. 9. The vascular system in an instance of chronic bronchiectasis of saccular type. In this photograph the pulmonary arteries and veins and the bronchial arteries and veins have all been injected. The bronchial veins have received their injection from a pulmonary vein by means of a short, stout branch seen leading downward from the vein labeled V. This short, stout segment can be considered the straight limb of a T, one branch of which proceeds proximally to enter the hilar network of bronchopulmonary venules. The proximal end of this is destined for the branches of the systemic venous system within the mediastinum. The distal branch proceeds longitudinally along a bronchus and is grossly visible much farther peripherally than is true under normal circumstances. The enlarged bronchial arteries appear black.

FIG. 10. The relations shown in the photograph of Figure 9 are simplified in this drawing. The pulmonary vein is labeled V to correspond with the vessel shown in Figure 9. The dark vessel is a pulmonary artery. Bronchial arteries are not shown in the drawing.

FIG. 11. Pulmonary emphysema. Case 1. This is a posterior view of the cast showing one bronchopulmonary venous plexus proceeding upward from the superior segmental pulmonary vein of the left lower lobe (V^6 of Boyden[20]) to be distributed upon the left main bronchus, pulmonary artery (P.A.), and the adjacent hilar structures. From the inferior margin of V^6 there is derived an elongated plexus which proceeds downward and which branches into numerous narrow, twisted vessels that in turn communicate with a complex plexus derived from the azygos vein. The latter appears dark in the photograph and loops over the right main bronchus.

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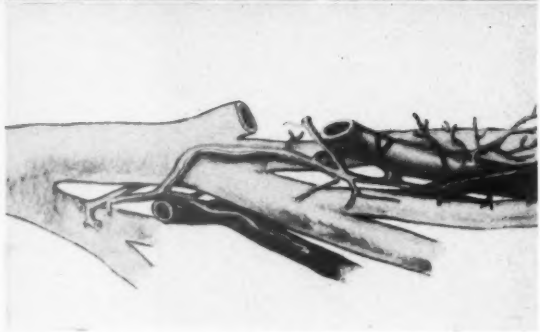

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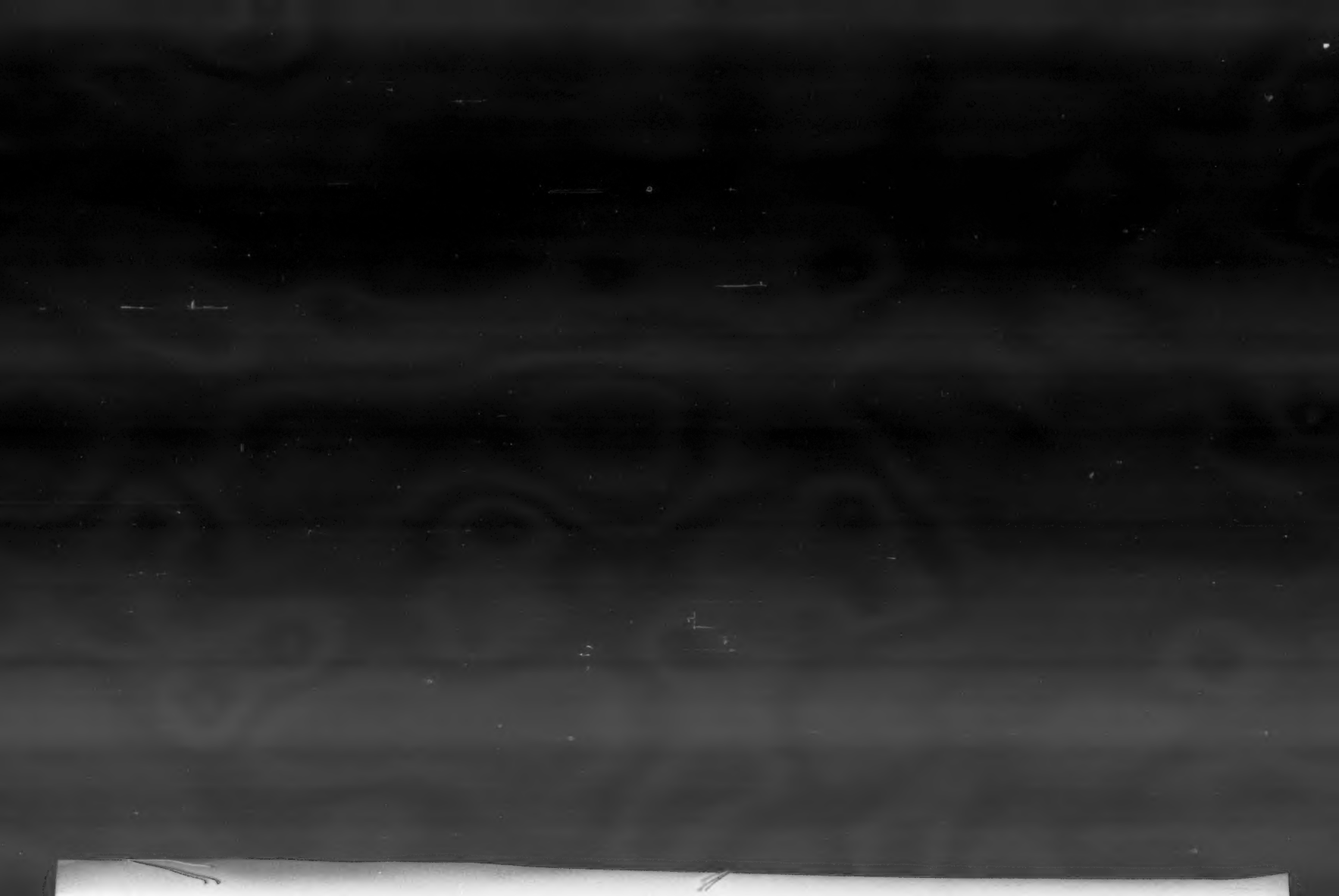
FIG. 12. Pulmonary emphysema. Case 2. A photograph of a drawing showing a large bronchopulmonary vein derived from the inferior vein proceeding upward to join a mediastinal plexus that then is drained on the posterior aspect into the azygos vein. The latter is seen behind the right bronchus that is pictured as if semi-transparent. Other elements of the bronchopulmonary venous plexus are seen as branching vessels on the anterior aspect of the middle and lower lobe bronchi.

FIG. 13. Posterior view of the cast illustrated in Figure 12. The vessel that joins the mediastinal plexus to the azygos vein is demonstrated beneath the carina. Bronchopulmonary veins that embrace bronchi of the right side are seen to join the mediastinal plexus. Enlarged bronchial arteries appear black.

FIG. 14. Pulmonary emphysema. Case 3. A general view of the bronchovascular cast. The branch extending upward from the superior vein (S.V.) and arching medially is demonstrated. There is also the very large, twisted mass of pleural vessels that loops over all of the hilar structures and enters an immense subcarinal plexus from below. The azygos vein (Az) also is visible just to the right of the right main bronchus as a bulging sausage-shaped structure. For comparison with Figure 17. I.V. = inferior vein. P.A. = pulmonary artery.



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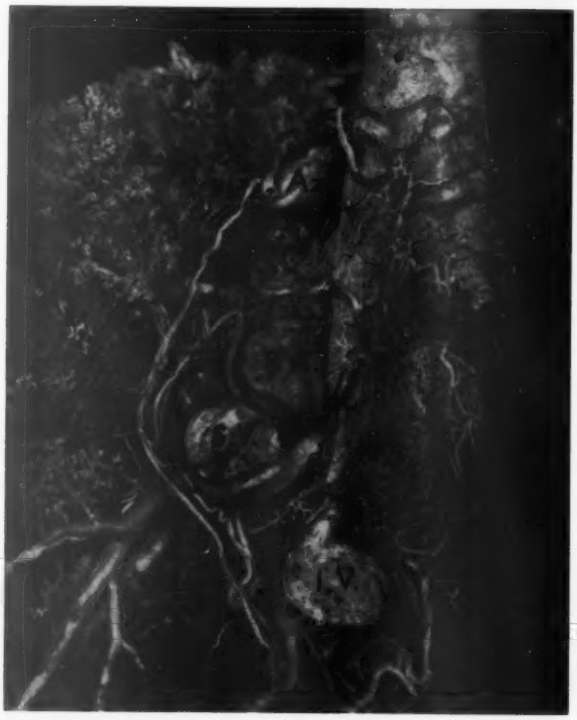
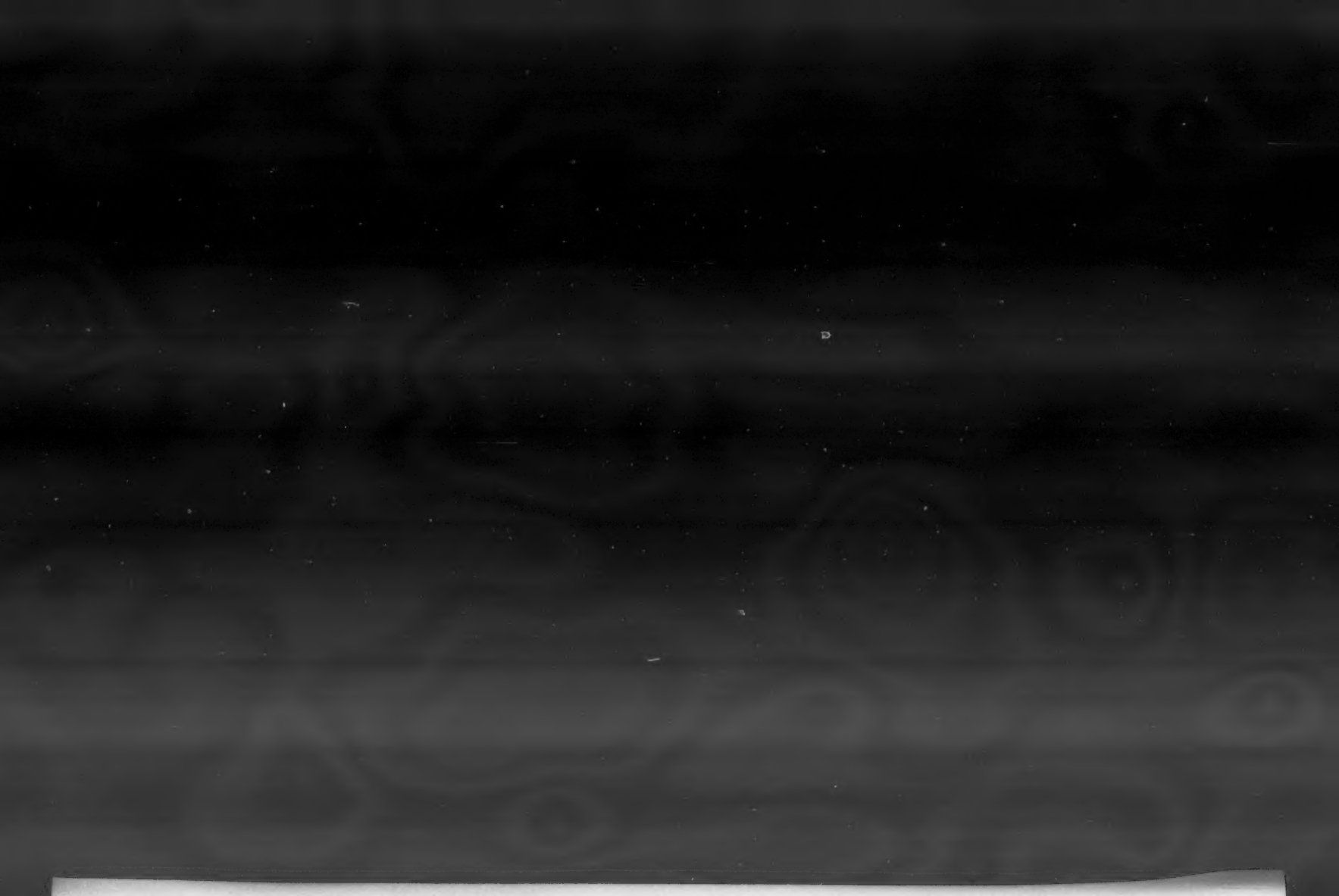


FIG. 15. One of the vessels draining the subcarinal plexus is seen entering the azygos vein on the inferior aspect of the latter. The point of entrance is indicated by the lowermost arrow (no. 3) in Figure 17. The azygos vein has been injected with a pale-colored material and has been transected; it appears as an irregular mass of white material just beneath the carina and to the right. Enlarged bronchial arteries appear black.

FIG. 16. Additional vessels are seen entering the azygos vein from the left side. Their points of entrance are indicated by the two upper arrows (nos. 1 and 2) in Figure 17. These are likewise draining vessels of the large subcarinal plexus that is seen at a slightly different angle from that employed in making the photograph of Figure 15. Enlarged bronchial arteries appear black.

FIG. 17. A drawing showing the major connections of the various bronchopulmonary venous plexuses in case 3. The right superior vein (S.V.) yields a stout branch that proceeds upward and then enters the subcarinal plexus. The pleural plexus and its connections inferiorly with the subcarinal plexus also are shown, as well as its circumferential course to its ultimate destination in the azygos vein in the region of the uppermost arrow (no. 1). The other arrows shown in relation to the azygos vein demonstrate additional connections which are pictured in Figures 15 and 16. For purposes of the drawing, the complex and extensive subcarinal plexus has been greatly simplified. I.V. = inferior vein.



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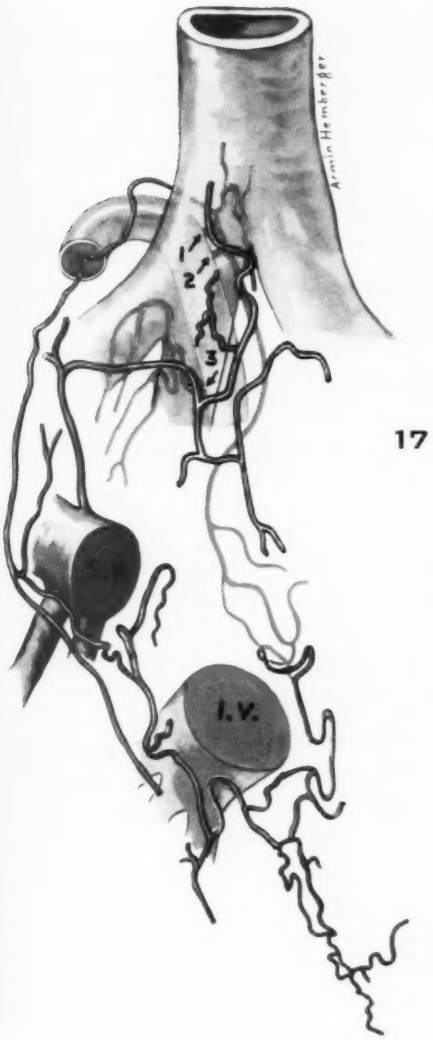

FIG. 18. Pulmonary emphysema. Case 4. This represents an anterior view of the left lung showing the origin of bronchopulmonary veins from the anteromedial branch of the inferior vein (I.V.) and also from branches of the upper lobe veins on the anterior aspect of the lung. These are described in the text.

FIG. 19. Posterior aspect of cast shown in Figure 18. The very large bronchopulmonary venous plexus derived from the superior segmental vein of the lower lobe (V^6) is shown proceeding directly upward posterior to the hilar structures. A very large, tortuous vessel ending above the cut surface of the left main bronchus is destined for the accessory hemi-azygos vein. Other extensive bronchopulmonary venous plexuses are seen on the medial aspect in the vicinity of the pulmonary ligament, apparently applied to other branches of the inferior vein (I.V.).

FIG. 20. Pulmonary emphysema. Case 5. An immense plexus of fine bronchopulmonary veins is visible on the medial aspect of the right lung. Beneath the medial segmental bronchus of the middle lobe (labelled B^5) is seen a T-shaped vein connected with a large pulmonary vein. This extends some branches peripherally and others proximally. A direct continuation can be traced from a third order branch of the medial segmental bronchus to the hilar plexus.

FIG. 21. Drawing of the region described in the legend for Figure 20. The branches of the T vein are clearly seen.

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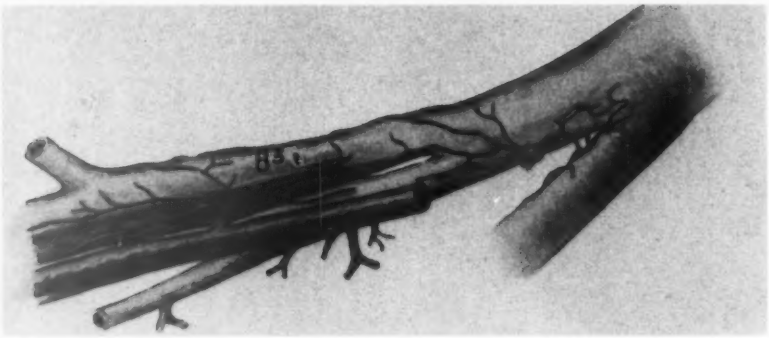

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FIG. 22. Pulmonary emphysema. Case 6. Close-up of superior segmental bronchus of left lower lobe showing peripheral venules derived from the pulmonary veins distributed upon the walls of the bronchi. Their intimate relation to the wall of the bronchus and their plexiform character distinguish them as bronchopulmonary venules. Extending toward the medial aspect is an extensive plexus of larger bronchopulmonary veins, elements of which are connected with the intimate bronchial system. The latter are also connected with true pulmonary venules.

FIG. 23. Pulmonary emphysema. Case 6. Tortuous pleural venules forming a dense plexus injected from the peripheral ends of pulmonary venules.

FIG. 24. Pulmonary emphysema. Case 7. Drawing indicating the origins of large bronchopulmonary venous plexuses and their junction in an extensive subcarinal plexus. It is apparent that all of the major pulmonary veins are placed in communication by this system of vessels. The x marks in the drawing indicate breaks in the vascular cast resulting from artefact.

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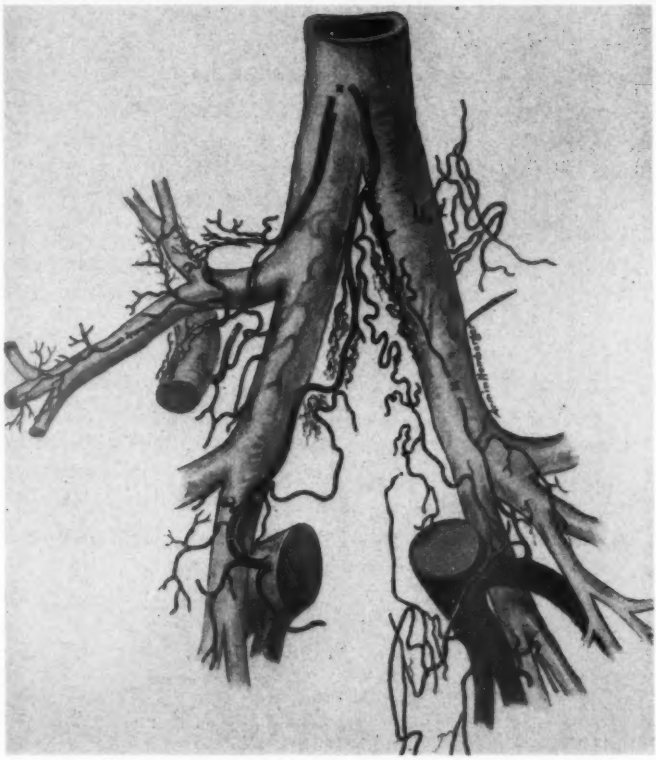

MORPHOLOGIC STUDY OF CANINE LUNGS AFTER LIGATION OF THE PULMONARY VEINS *

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Walsh,[1] in 1907, attempted ligation of the pulmonary veins in dogs but the experiment was unsuccessful owing to inadequate surgical technique which led to acute massive collapse of the lung. Tiegel,[2] O'Shaughnessy,[3] and other investigators[4,5] ligated certain lobar veins as a therapeutic measure in experimental or clinical pulmonary tuberculosis. As the results of this surgical manipulation were equivocal, the procedure was abandoned and related pathologic effects remained uncharted. Without reporting detailed pathologic studies, Hyndman and others[5] stated that ligation of the left pulmonary vein in dogs was "not in the least incompatible with life." Kent and Blades[6] and Brantigan,[7] in surgical investigations, implied that gangrene of a lobe might occur if the pulmonary vein were compromised during a thoracic operation. In 1948, Swan and Mulligan[8] reported two instances of complete ligation of the pulmonary veins on the right and left side in the dog; the induced congestive state was rapidly fatal.

Because of current interest in the circulation of the lung in chronic cardiac and pulmonary disease, experimental occlusion of the entire pulmonary venous system of one lung in the dog was attempted and a long-term study concluded.[9] The morphologic changes in the lungs of such animals are the subject of the present report. Physiologic studies and the effects of combined arterial and venous occlusion are to be presented in subsequent contributions.

The principal purpose of this communication is to correlate the effects of pulmonary venous occlusion on the pulmonary parenchyma as a contribution to an understanding of pulmonary infarction.

MATERIALS AND METHODS

Healthy adult mongrel dogs, varying in weight from 6 to 14 kg., were used. Under endotracheal anesthesia, the pulmonary veins were exposed at the hilum and divided between transfixion ligatures. This maneuver prevents recanalization of the vessels. Aseptic "silk" technique was used. At the conclusion of the operative procedure, each animal was given a subcutaneous injection of normal saline solution amounting to 30 cc. per kg. of body weight. No further supportive

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therapy was administered except in certain animals which received penicillin.

Twenty-five animals were subjected to complete ligation of pulmonary veins from one lung; the procedure was carried out in 17 dogs on the right side and in 8 on the left. About one half of the animals in each series received 20,000 units of penicillin per kg. of body weight, administered daily for a period of 3 days.

Animals in both series were sacrificed at intervals from 24 hours up to 1 year after operation. The heart and lungs were removed *en bloc* and the lungs were injected through the trachea with formalin solution and fixed in a formalin bath. Multiple blocks from each lung were sectioned at 6 μ and sections from all lobes were stained by the following methods: hematoxylin and eosin, Masson's trichrome, Turnbull's Prussian blue, French-Weigert's elastic tissue, Lendrum's phloxine-tartrazine, and Reinhardt's modification of the periodic acid technique.

Results

Clinical Course. The 8 dogs in which all of the pulmonary veins of the left lung were ligated and severed were divided into two groups. Four dogs received penicillin, 4 did not. All 8 animals survived the operation.

Of 9 dogs subjected to division of all pulmonary veins on the right side without postoperative penicillin, 6 died in 3 days or less. A second group of 8 dogs undergoing the identical operation received penicillin for 3 days. In contrast, all but one of these animals survived and were subsequently sacrificed. The single death occurred in association with inundation of the unoperated lung by large amounts of bloody, mucoid sputum which produced atelectasis and ventilatory insufficiency. All of the animals with venous occlusion produced bloody sputum in varying amounts; some dogs managed to cough it out more effectively than others.

Bacteriologic Study. In the series of animals in which the right pulmonary vein was ligated without postoperative penicillin therapy, positive cultures were obtained from the blood and lung in the 6 animals which died. The organisms obtained on culture represented a broad bacterial spectrum, being hemolytic streptococci, pneumococci, and unidentified Gram-negative bacilli. Anaerobic cultures were consistently negative.

In all of the animals receiving penicillin, irrespective of the side on which the veins were ligated, only one showed a positive blood culture; this animal's blood was subsequently negative on repeated cultures.

MORPHOLOGIC ALTERATIONS

Venous Occlusion of One to Three Days' Duration

At necropsy, 1 to 3 days after venous occlusion, the lung on the operative side was grossly enlarged, deep plum purple, and resembled liver (Figs. 1 and 3). Histopathologic studies revealed diffuse extravasation of red blood cells and eosinophilic protein fluid into all alveolar sacs of all lobes (Fig. 2). Even after 3 days of congestion, the septal cells were still distinguishable. There were no demonstrable areas of necrosis (Fig. 2). The inflammatory response was confined to the larger vascular channels. In the subpleural zone, the septal capillary congestion was less pronounced. There was an early deposition of eosinophilic fibrin.

The majority of red cells, though extravasated, still retained their corpuscular outline. In a few areas, there were scattered beginnings of what appeared to be a fibrinous bronchopneumonia. In these areas bacterial stains were consistently negative for organisms. Some areas, particularly in marginal and basal portions of the lung, showed a greater concentration of red blood cells than other more central zones. As early as 24 hours after venous occlusion, networks of fibrin traversed the fluid-filled air sacs. At this phase, there was no great increase in migration of septal cells. No swollen or cuboidal septal cells were noted. With Lendrum's phloxine-tartrazine stain, the alveolar walls were apparently intact.

Some of the larger channels, particularly venous, were filled with packed red cells; the others were empty. No established thrombotic occlusions were noted. This complete patency of large vascular channels beyond the point of ligature has been demonstrated also by Schlaepfer[10] in experimental ligation of the pulmonary artery.

Venous Occlusion of Ten to Twenty-one Days' Duration

At 10 to 21 days there was early clearing, resolution, and aeration in the lower lobe (Figs. 3 and 4) but the remainder of the lung remained dark purple, with small areas of atelectasis. It was estimated that approximately one fourth of the lung had cleared (Fig. 4). In the remaining portion of the lung, there were innumerable ghosts of red cells, groups of intact red blood cells, clumps of protein, eosinophilic fluid, and fibrin within the alveoli. The remainder of the lung showed focal areas of pronounced increase in septal cells with an occasional air sac delimited by these cells arranged in a syncytial pattern. Associated with the increase in septal cells there were masses of fibrin and disintegrated red cells incarcerated in the septal cell buds (Fig. 8).

As early as the tenth day, hemosiderin was found scattered throughout alveoli and also concentrated around large bronchovascular channels and lymphoid collections of the lung.

Throughout the lung the septa were swollen, mostly due to fluid and early fibrocellular proliferation. The subpleural zones showed a greater degree of clearing than other zones of the lungs. The fibrinous deposits on the lung surface (Fig. 5) showed early fibroblastic organization and apparent continuity between the vascularized pleura and the existent septal capillaries of the lung parenchyma.

Venous Occlusion of One Month's to Six Weeks' Duration

At 1 month, the lung was approximately two thirds aerated with the remainder marked by depressed areas of atelectasis. At this period there had been remarkable anatomical restitution of the lung except for the puckered blue areas of collapse scattered throughout, but particularly near the periphery (Fig. 6). This finding was exclusive of the pronounced operative adhesions between lung and pleura.

Microscopic examination at the edges of the enfilades of the early collagenous scar showed fused septal and intra-alveolar fibroblastic structures, somewhat reminiscent of the "bourgeons conjunctif" (Fig. 9) originally described by Masson in rheumatic pneumonitis.[11]

Hemosiderin was still present, although for the most part it was deposited in the bronchovascular connective tissue rays (Fig. 10) and the centers of the focal, septal, fibroblastic scars.

In scattered foci, French-Weigert's elastic tissue stain showed collapse of the elastic tissue skeleton with partial fraying of this tissue and with the fragments surrounded by early fibroblastic proliferative change.

Venous Occlusion of Five Months' to One Year's Duration

At 5 months to 1 year, the lung was approximately 90 per cent aerated. As the lungs lay *in situ,* sizeable collateral vessels could be seen to enter the lung through the thickened pleura. The red pleural plaques were particularly striking at the thoracotomy site (Fig. 7). Only a few focal scars remained, puckered in pattern, with the intervening lung parenchyma apparently intact (Fig. 12). The scars were made up of strands of acellular collagen and scattered fibrocytes. The alveolar walls showed slight interstitial fibrosis (Fig. 11) but for the most part these walls were unchanged (Fig. 13). On comparison, the septal capillaries on the operative side appeared to be more congested than those on the non-operative side. All traces of hemosiderin had disappeared from the lung.

The pulmonary veins and arteries, except in the region of the subpleural zone, were unaffected. In the few remaining linear scars and in the pleural adhesions, the larger vessels were slightly thickened due to subintimal fibroblastic proliferation.

In both series, a total of 25 dogs, the lung on the non-operative side was examined routinely and no pathologic alterations were seen.[12]

SUMMARY OF MORPHOLOGIC ALTERATION

The present experiments have shown that acute occlusion and ligation of the entire pulmonary venous system of the dog's lung result in profound septal congestion and intra-alveolar extravasation of blood into all lobes. The subsequent evolution of the process is as follows: Within 3 weeks to 3 months, the formed elements of the blood and fluid have been cleared from the lung. Co-existent with the process of clearance throughout the lung is the development of a few irregular, scattered, septal, fibroblastic scars along with fresh evidence of minimal interstitial fibrosis. Collections of hemosiderin are found in the bronchovascular units of the lung. The pleura is diffusely thickened owing to organization of a fibrinous exudate. Major and minor pulmonary veins are patent. The pulmonary arterial system is intact.

At the end of 3 months, the lung is well aerated and its form and texture are like those of the unoperated side. Only a few focal scars remain. The operative site shows prominent venous lakes and dense vascular adhesions, producing a broad, red, adherent plaque between the visceral pleura and the chest wall.

At the end of 1 year the lung almost completely fills the chest cavity. Only dense vascular adhesions at the operative site remain as evidence of the original injury.[1] Apart from a few persistent focal scars and the thickened septal walls the lung parenchyma is intact.

Discussion

Under the conditions of this experiment, total occlusion of the pulmonary veins from the dog's left lung is well tolerated. In contrast, the mortality is high with occlusion of the venous drainage from the right lung, although this mortality can be mitigated by administration of penicillin. It has been suggested in previous work on the subject that the greater mortality associated with right-sided ligation may be related to the relatively greater lung volume on this side. That infection may also play a rôle in the fatal outcome is suggested by preliminary bacteriologic studies. When bloody sputum from a consolidated lung on the operative side produces flooding and ventilatory insufficiency of the opposite lung, there is adequate reason for a fatal

termination from this factor alone. No evidence was found to suggest that acute cor pulmonale played a rôle in the failure of certain animals to survive the venous interruption.

The morphologic changes at various periods up to 1 year after occlusion have been detailed but the implications warrant further discussion. The clearance of the lung after the congestive episode may be brought about in three ways: through the bronchial tree, by lymphatics, and by phagocytic action. In the early phases of congestion the bloody fluid is carried by ciliary action and bronchial movement to the major bronchi. Tussic action completes the evacuation. As the elimination of fluid through the bronchi diminishes after the first few days, the rôle of lymphatic drainage is of increasing importance.[13] Phagocytic action is also a continuing process, although over-shadowed in the early phases by the gross bronchial and lymphatic elimination.

The later clearance of the lung may depend on circulation through the broad, widely vascularized adhesions between visceral pleura and chest wall at the operative site. These permit drainage into the systemic venous circulation. Our own observations and plastic injection studies by Ameuille and co-workers[4] emphasize the importance of this vascular connection. Along with the clearance of débris from the lung, there was a progressive expansion. The depressed and puckered areas gradually disappeared, since the majority of these foci were simply areas of non-aerated lung parenchyma. The remaining "scars" were for the most part due to septal cell proliferation which gradually disappeared over the course of several months, leaving only an occasional web of collagenous scar at the end of 12 months. No precise explanation was apparent for the scattered fibroblastic scars noted later in the course of the induced experimental state. These true scars may be a host response to retained lymphatic detritus following the phase of congestion, or evidence of some obscure injury antedating the experiment. The architecture of these isolated scars is unlike that described by Castleman[14] for healed infarcts.

One interesting aspect of the clearance problem concerns the hemosiderin accumulations which are scattered haphazardly throughout the lung. These deposits did not possess the size, shape, or constancy of situation which Lendrum[15] has reported in the lungs of patients with mitral stenosis. From our experiments, mere venous congestion was incapable of producing specific localization of iron deposits as found in the rat by Magarey.[16] Evidence from our experimental studies rejects such a belief and indirectly supports Lendrum's observations that the repetitive distribution of siderotic deposits is probably related to continuing differences in intravascular pressure at anastomotic sites.

Such a situation occurs in the congested lung of mitral stenosis with iron pigment accumulating in the respiratory bronchiole—the site of anastomosis between the pulmonary and bronchial circulations.

Recent investigations[12,17,18] indicate that occlusion of the bronchial artery produces pulmonary infarction, but Ghoreyeb and Karsner[19] earlier had shown that occlusion of the pulmonary artery required the additive component of congestion to induce necrosis of the lung. Our observation shows that pulmonary venous occlusion alone is incapable of provoking infarction.

Summary and Conclusion

The pulmonary veins from the right or left lung were divided in 25 dogs. Division of the left pulmonary veins did not result in death. In contrast, the mortality was high when the right pulmonary veins were divided; this mortality was obviated by the postoperative administration of penicillin. The greater severity of reaction following ligation of the right pulmonary veins may be partially due to quantitative differences in venous flow on the two sides.

Anatomical studies after venous interruption showed intense and diffuse capillary congestion and extravasation of blood into the lung.

Anatomical study of the lungs following ligation of the veins revealed rapid clearance of the blood constituents within 6 weeks, without evidence of infarction. At the end of 12 months there was complete expansion of the lung and except for the occasional linear scar and operative adhesions, there was no morphologic evidence of damage to the lung.

The lack of permanent microscopic changes in the lungs following pulmonary venous ligation indicates that interference with outflow through the pulmonary veins in dogs does not lead to devitalization.

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LEGENDS FOR FIGURES

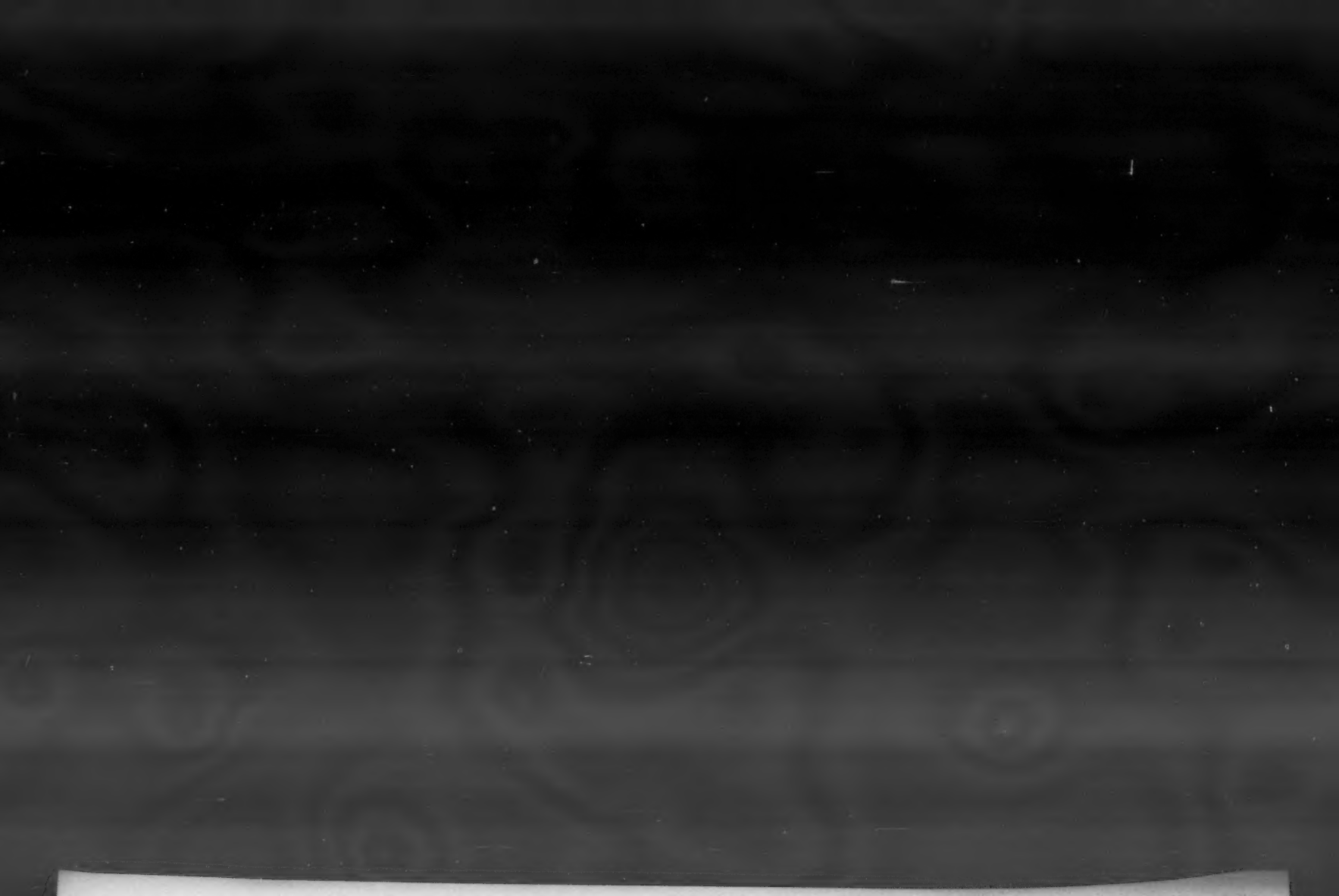
FIG. 1. Gross photograph of the lungs and heart with all right pulmonary veins ligated and divided. Advanced congestion of all lobes of the right lung.

FIG. 2. Lung 12 hours after experimental venous occlusion, showing early filling of alveoli with red cells and migration of leukocytes. Hematoxylin and eosin stain. × 120.

FIG. 3. Transections of lungs on the venous occluded side. Deep red-brown congestion after 24 hours of venous congestion (specimen on left); center specimen shows considerable clearing of the lung after 10 days; specimen on the right shows thickened pleura, dilatation of bronchioles, and almost complete clearing of the lung after 6 weeks.

FIG. 4. After 10 days of venous occlusion, the lower lobe of the right lung is almost cleared; early vascular pleural adhesions may be noted.

FIG. 5. Lung, showing, after 10 days of venous occlusion, prominent fibrinous pleuritis with the subpleural zone containing intra-alveolar erythrocytes, septal cell proliferation, and beginning hemosiderosis.



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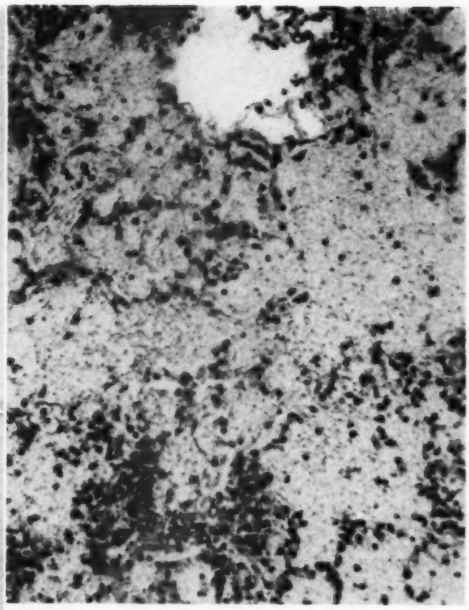

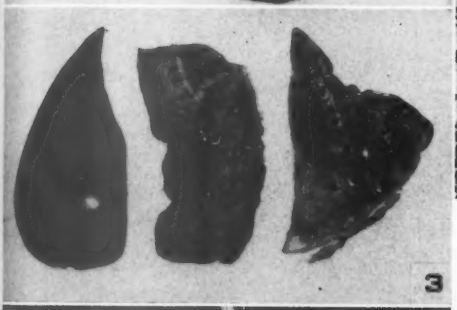

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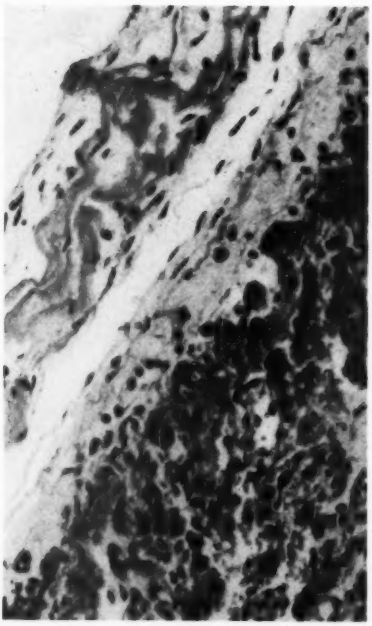

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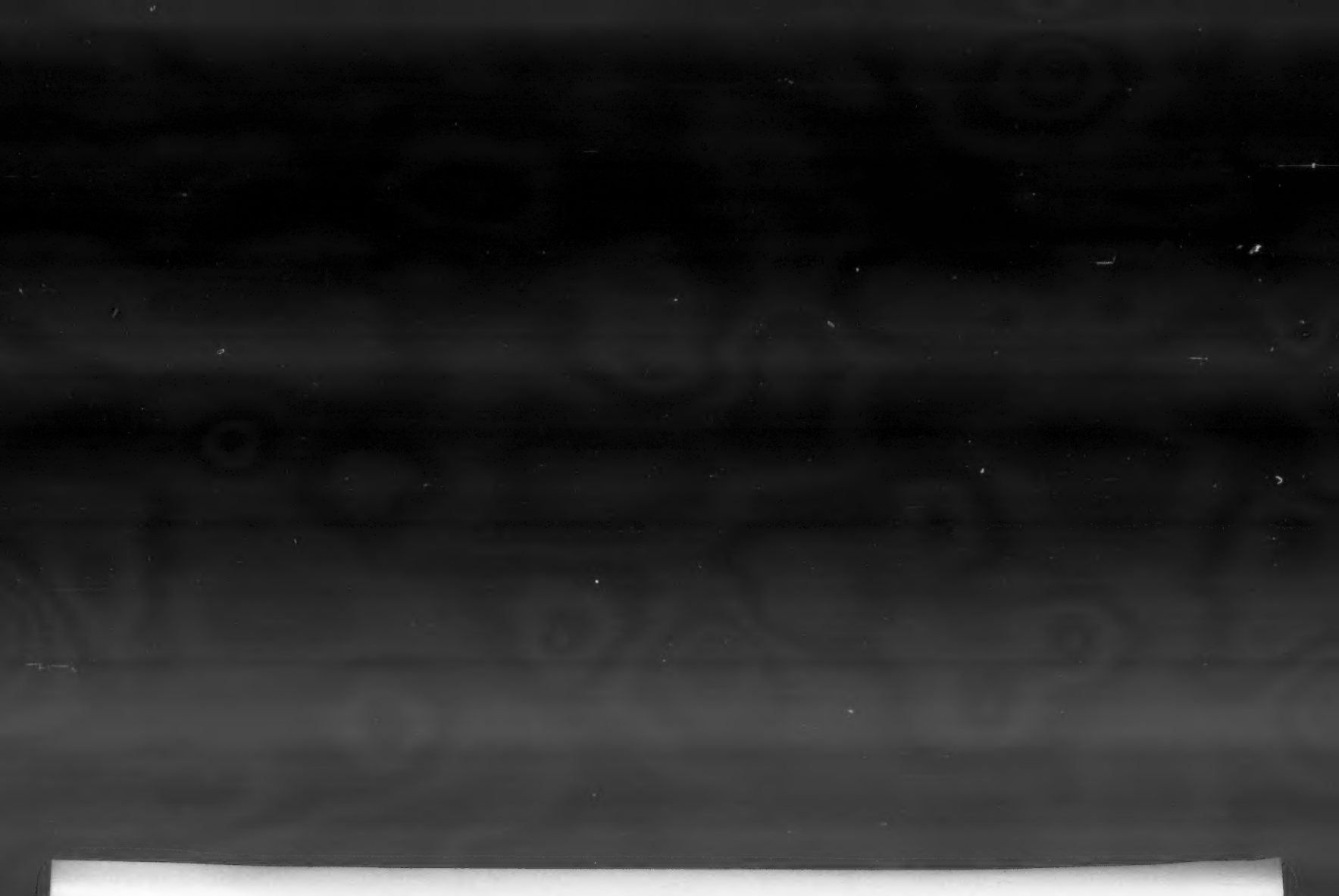
FIG. 6. After 1 month, lung on the ligated side shows wide regions of clearing and persisting areas of atelectasis and congestion.

FIG. 7. At 3 months, the lung on the ligated side shows only a broad adherent scar at the apex of the lung.



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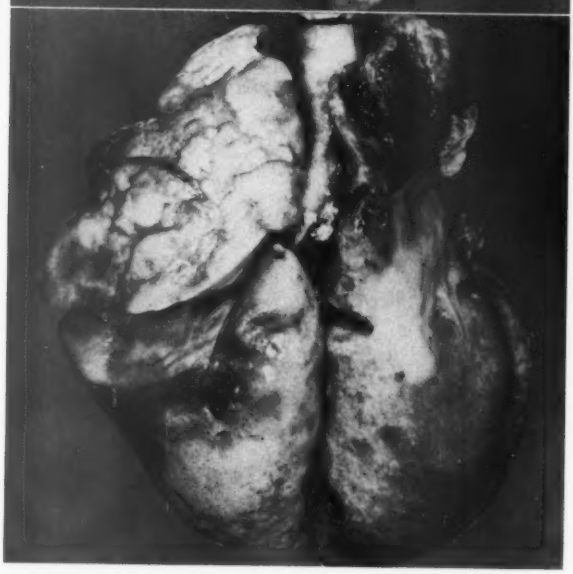


FIG. 8. Septal organization after 10 days of venous occlusion (left lung), with red cells incarcerated in broadened septa, septal cell proliferation and budding into alveoli. Hematoxylin and eosin stain. × 240.

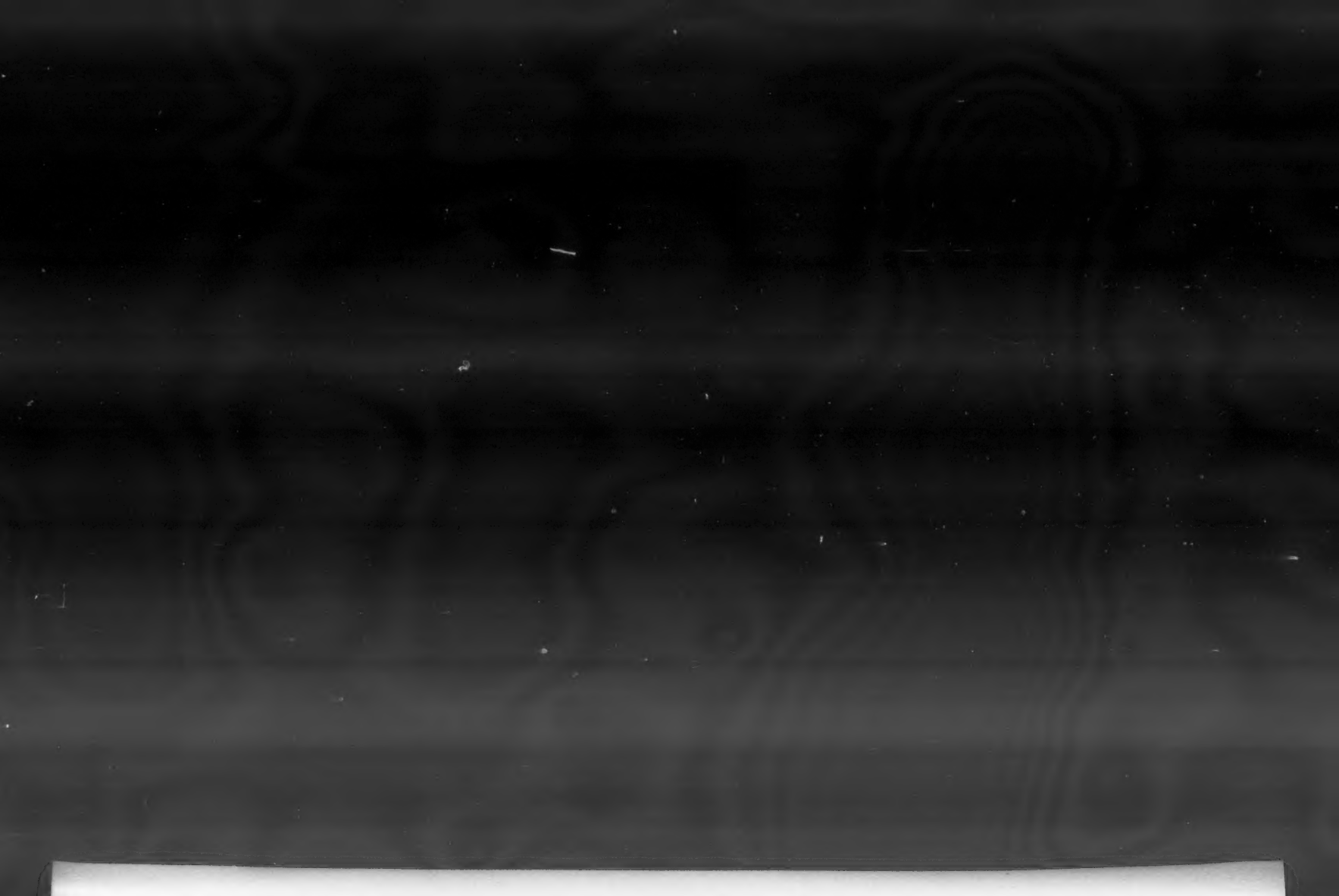
FIG. 9. Six weeks after operation, with early interstitial fibrosis and intra-alveolar buds of septal and fibroblastic elements simulating "rheumatic fever buds" of Masson. Hematoxylin and eosin stain. × 240.

FIG. 10. Collections of hemosiderin are prominent in the bronchovascular walls at 10 weeks after operation. Hematoxylin and eosin stain. × 320.

FIG. 11. Scattered foci show fine interstitial fibrosis. Hematoxylin and eosin stain. × 420.

FIG. 12. Three months after ligation of the veins, irregular stellate scars were noted throughout the lung. Intervening lung parenchyma shows factitious emphysema and limited foci of interstitial fibrosis. Lendrum's phloxine-tartrazine stain. × 120.

FIG. 13. Lung parenchyma from the ligated right lung 1 year after operation, showing parenchyma free of pathologic alterations. Hematoxylin and eosin stain. × 180.



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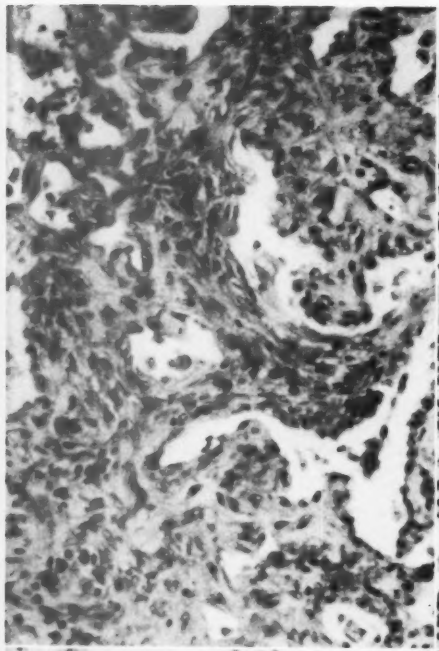

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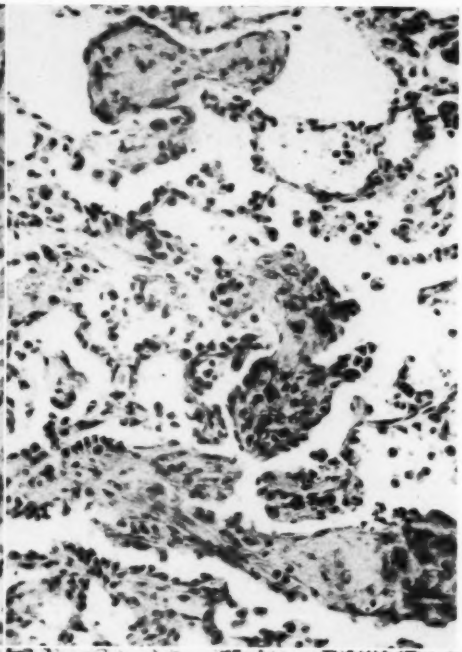

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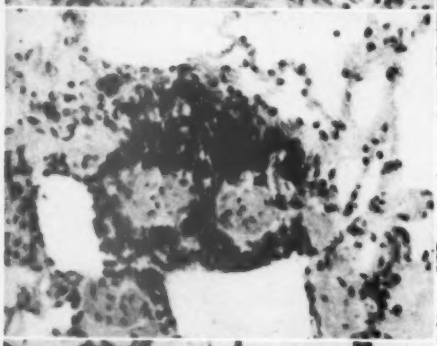

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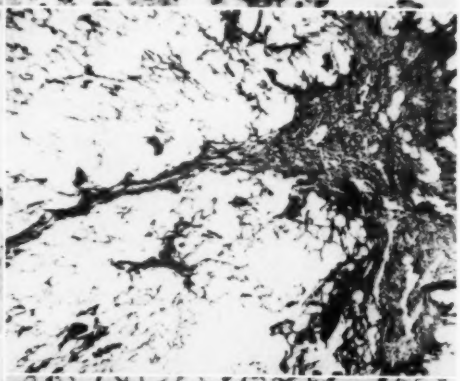

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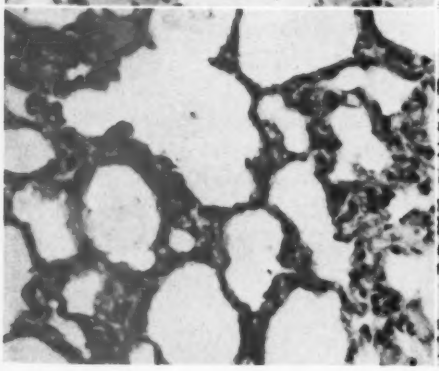

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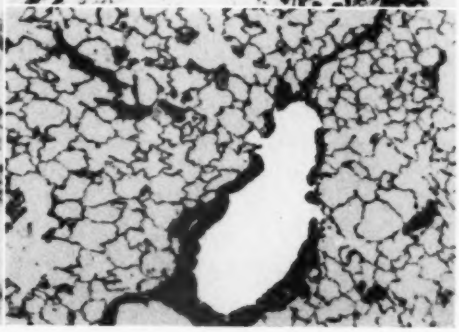

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THE FATE AND PATHOLOGIC EFFECTS OF PLUTONIUM METAL IMPLANTED INTO RABBITS AND RATS *

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Previous toxicologic and metabolic studies with plutonium have been carried out for the most part with the citrate and nitrate salts of this element in solution.[1-3] Unlike radium and strontium, which concentrate primarily in the skeletal tissues, considerable amounts of plutonium are retained in liver, spleen, and bone marrow, as well as in the bones. The mechanism by which plutonium is bound to the tissues is not well understood in its details, but it is evident that it is very firmly incorporated, for very little is excreted in the months and years following injection. Urinary and fecal elimination contribute so little to the loss of plutonium from the body after a short initial period of high excretion that the total retained amount is about 70 to 80 per cent of the injected dose. The radiotoxicologic implications of this high rate of retention are obvious, and some late effects have been described previously.[4,5] It was of interest to inquire to what extent plutonium would be absorbed from the subcutaneous tissues when implanted in metallic form and whether it would follow a pattern of metabolism similar to that of the salts of plutonium in solution. It was of further interest to study the local tissue reactions at the site of implantation as well as other systemic reactions due to the absorbed plutonium.

EXPERIMENTAL PROCEDURES

Plutonium ($_{94}Pu^{239}$) is a bright silvery metal which readily tarnishes on contact with air with the formation of a black coating. This film has been identified as PuO; this eventually becomes the stable PuO_2. The metal had been received in the form of a thin wire, approximately 1 mm. in diameter. Pieces of metal, ranging in weight from 0.61 to 1.80 mg., were implanted with the aid of a trochar under the skin of 8 male rabbits and 3 female rats. The relatively small amount of metal available for biologic experimentation precluded the use of more animals in this experiment. The metal fragments were lodged subcutaneously in the left upper quadrant of the abdomen, below the left costal margin. The hair in this area had been shaved prior to implantation, and this facilitated the gross inspection and palpation of the implantation sites. The animals were examined regularly both by palpation and roentgenographically. They were permitted to live out their life span

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and were sacrificed only when moribund. The organs were prepared and weighed for chemical analysis and samples were taken for histologic examination when indicated. Those taken for analysis included the liver, spleen, kidneys, lungs, and testes. The following bones were analyzed separately in the rats: femur, humerus, tibia, rib, and spine. In the rabbits, only the femur was analyzed, and the plutonium content of the remainder of the skeleton was then calculated by multiplying the concentration of plutonium found in the femur by a factor of 20. This method of estimating the plutonium content of the skeleton has been found to be reliable in previous experiments.[6] In all cases the site of implantation was removed with a wide margin of normal tissue and analyzed. After weighing, the tissues were ashed in an electric muffle furnace at 550° C. for 24 hours, following a preliminary drying at 150° C. for 12 hours. Analysis for plutonium was then carried out by the lanthanum fluoride method.[2] Urine and fecal samples were treated by wet ashing, and the residue was taken up in a small volume of 2N HNO_3; plutonium determination on these samples was carried out by the same method. The amount of plutonium present in the samples was estimated by use of a pulse ionization chamber in combination with a 300,000-fold amplifier and a scale-of-64 circuit. The instrument operated at 50 per cent geometry with an additional 4 per cent backscatter from the platinum disk, giving a counting yield of 52 per cent.

Excretion Studies in Rats

No plutonium was found in the urine and feces of rats during the first 10 weeks following implantation. After wet ashing, weekly urine samples were counted in their entirety; only aliquots of the fecal samples were analyzed. A second collection of urine and feces was made 15 weeks after implantation. At this time both contained measurable amounts of activity. The average count of the urine samples was 71 counts per minute per week (0.001 μg.). Fecal excretion was considerably higher, averaging almost 3000 counts per minute per week (0.044 μg.). A few additional excretion studies were carried out subsequently on daily samples of urine and feces and the results were essentially identical with the findings on the weekly pooled samples taken earlier. A final determination was made on 2 animals 10 months after the implantation, and it was found that the excretion had diminished to an average of only 22.5 counts in the urine and to 217.5 counts in the feces.

Excretion Studies in Rabbits

There was no detectable excretion of Pu in either the urine or the feces of rabbits during the first 2 months of observation. A few urine

samples were taken again at 4 and at 10 months and these showed barely measurable amounts of activity, measured on a low background alpha counter; they were only slightly above background. No plutonium was found in the feces.

Absorption and Distribution

The average amount of plutonium absorbed from the implantation site in the two groups of animals was found to be less than 0.5 per cent, with a range from 0.09 to 1.20 per cent (Table I). Data on the

TABLE I

Plutonium Absorption in Rabbits and Rats

	Days after implantation	μg. Pu implanted	Total Pu absorbed, μg.	Per cent absorbed	Size of reaction zone at death (mm.)
Rabbits	2	1800	None	None	3 x 2
	260	1000	1.63	0.16	9 x 5
	340	1330	8.43	0.63	61 x 22
	516	670	1.47	0.22	11 x 8
	623	770	5.87	0.76	53 x 24
	710	1140	13.67	1.20	7 x 4
	738	1250	4.56	0.37	63 x 21
	1048	720	1.97	0.27	29 x 27
Rats	356	800	2.27	0.28	4 x 3.5
	484	610	1.11	0.18	3 x 2.5
	580	740	0.70	0.09	4.5 x 3.0

animals in Table I are arranged in order of survival time, and the dimensions of the local tissue reaction to the implanted piece of metal are given in the last column, showing the measurements at the time of necropsy.

In the rabbits there was a wide variation in the amount of absorbed plutonium, ranging from 1.47 to 13.67 μg. per animal. No plutonium was found in the tissues of the animal that died on the second day. There was no correlation between the amount of plutonium originally implanted and that found at death. Moreover, the surface area of the implantation site attained at death appeared to bear no relation to the fraction of plutonium that was found to have been absorbed from this site. Survival seems to have had no influence on absorption. In the rats the retention values were 0.70, 1.11, and 2.27 μg. per animal. It is of interest that it was the animal with the largest amount of plutonium that developed a tumor of the spine. The percentage absorption in the rabbits was about three times that in the rats (0.52 and 0.18 per cent, respectively). The factors governing the greater absorption from the site of implantation in this species are obscure.

The pertinent data concerning the distribution of Pu in the skeleton

and liver are shown in Table II. Analysis of these data reveals a striking difference between the two species with respect to the amount of Pu taken up by the skeleton and liver. With amounts of Pu expressed in per cent of the total absorbed dose, the following values were found: rabbit liver 55 per cent and skeleton 44 per cent; rat liver 6 per cent and skeleton 85 per cent. On the basis of specific activity, *i.e.*, Pu per gram of wet weight, the concentration of Pu in the liver of the rabbits exceeded that of the bones by a factor of almost six, whereas the reverse was the case in the rats in which the concentration in the bones was five times greater than that in the liver. On the same basis, the skeleton of the rat contained on an average almost ten times more plutonium than that of the rabbit.

TABLE II

Organ Distribution of Plutonium Following Subcutaneous Implantation of the Metal

	Survival days	Skeleton			Liver		
		μg. per organ	μg./gm.	Per cent absorbed dose per organ	μg. per organ	μg./gm.	Per cent absorbed dose per organ
Rabbits	260	0.604	0.002	37.06	1.02	0.010	62.58
	340	3.38	0.009	40.09	5.02	0.051	59.55
	516	0.92	0.003	62.31	0.54	0.008	36.74
	623	1.78	0.005	30.32	4.05	0.051	68.99
	710	5.30	0.018	38.77	8.13	0.125	59.47
	738	2.40	0.007	52.63	2.09	0.027	45.83
	1048	1.88	0.007	95.43	0.05	0.003	2.54
Rats	356	1.75	0.109	77.09	0.21	0.027	9.25
	484	0.87	0.044	78.39	0.02	0.004	1.80
	580	0.69	0.028	98.57	0.04	0.005	5.71

Clinical and Roentgenographic Observations and Pathologic Findings

During the first few days the implantation sites were readily seen as slightly raised prominences under the skin. On palpation they were firm nodules, a few millimeters in diameter, and the overlying skin was freely movable in most instances. Four to 6 days after implantation, the nodules, in the rabbits, became progressively smaller and the prominences began to flatten out. It was impossible to locate the deposits with any certainty in the majority of the rabbits approximately 3 weeks after implantation, although some of them became palpable again later on. In contrast, the implants in the rats remained slightly elevated, firm and nodular throughout. Neither the rabbits nor rats ever showed a local skin reaction in the form of erythema or edema at the site of the implant. The regrowth of hair was not impaired and there was no change in texture or color of the regrown hair. One rab-

bit died on the second day following the implantation with acute enterocolitis of undetermined etiology. The site of the deposit presented the following picture at necropsy: Loosely attached to the subcutaneous tissues and overlying the abdominal muscles there was a small deposit of black granular material, confined to an area measuring 3 mm. in largest dimension. There was slight engorgement of the surrounding blood vessels but no generalized tissue reaction was seen. It was obvious that the metal piece had disintegrated into numerous very

TABLE III

Roentgenographic Measurements of Implantation Sites in Rabbits and Rats

	Survival time in days	2 weeks after implantation	At death
		mm.	mm.
Rabbits	2		3 x 2
	260	11 x 2	9 x 5
	340	12 x 5	61 x 22
	516	9 x 2	11 x 8
	623	3 x 5	53 x 24
	710	2 x 5	4 x 7
	738	4 x 6	63 x 21
	1048	11 x 2	29 x 27
Rats	356	3.0 x 3.5	3.5 x 4.0
	484	3.0 x 2.0	3.0 x 2.5
	580	2.5 x 3.0	3.0 x 4.5

small black granules, largely confined to the original site of deposition. Figure 1 shows the deposit as it appeared on the inner surface of the skin, and the corresponding roentgenograph is seen in Figure 2. (In the process of preparation, the skin folded over the deposit momentarily and part of the deposit was accidentally dislodged, thus giving the erroneous appearance of two deposits.)

Roentgenographs taken 2 weeks after implantation showed that the solid pieces of metal had broken into small fragments measuring from fractions of a millimeter to about 1 mm.; they were dispersed over a comparatively small area. This, however, measured more than the area originally occupied by the metal and in 7 rabbits it had a mean diameter of 8 by 3 mm. In the rats, the dispersion was more uniform and discrete, and the total space occupied was considerably smaller, measuring on the average 3.0 by 2.5 mm. (Table III). Subsequent pictures showed the persistence of some granules in all rabbits until death; however, it was not possible to trace any one fragment through a series of pictures since changes occurred continuously in them (Figs. 7 to 9). Concomitant with fragmentation of the metal there developed a localized and gradually increasing soft tissue density, producing an initially very pale, yet clearly visible shadow outlining the site of im-

plantation (Fig. 8). This soft tissue shadow showed considerable alteration in size, density, and general configuration in subsequent months (Figs. 6 and 9). These alterations were at least as conspicuous as those affecting the metal fragments and they were observed in 4 of the 7 rabbits. The other three implantation sites remained relatively stationary and small throughout, though all showed evidence of calcification. Although fragmentation of the metal also occurred in the rats, subsequent changes in the implantation sites were not very striking. In fact, the fragments appeared practically unchanged. A very small rim of a soft tissue shadow was noted, but it always remained stationary and well localized (Fig. 14).

Implantation Sites of Rabbits

Except for size, the general nature and gross appearance of the tissue reaction that had taken place around the implanted metal was quite similar in all animals. The deposits were found to be firmly attached either to derma or muscle and they presented themselves as flat deposits, 2 to 4 mm. in thickness, showing a pale to deep yellow material of very firm consistency, resembling calcified plaques. The surface of the plaques was smooth and uniform and the marginal outlines were sharp. The zone of demarcation, although often quite irregular, was clearly visible in all instances against the normal surrounding tissue. The adjacent connective tissue was well but not excessively vascularized. The following is a description of one of the smaller deposits from one of the rabbits: The implant consisted of a rounded, well demarcated and elevated plaque, firmly attached to a piece of abdominal muscle. It measured 9 by 5 mm. and was a pale yellow-gray. There was a slightly elevated central portion, and a few black-green granular deposits could be seen just underneath the surface (Fig. 3). The roentgenogram of this lesion showed a number of coarse granular shadows occupying about two thirds of the implant. The peripheral portion showed a more homogeneous and paler shadow, presumably consisting of calcified material, and a few small granules were found to be scattered about in the periphery (Fig. 4).

In contrast, the following is the description of an implantation site from a rabbit in which considerable enlargement had taken place: The area of reaction measured 53 by 24 mm. and consisted of a large, triangular, elevated plaque with irregular outlines and a generally smooth, though somewhat nodular, surface. The lesion was joined at the base of the triangle by edematous strands of connective tissue and it was densely adherent to the underlying abdominal muscle (Fig. 5). Periodic roentgenograms of this implant during life showed the follow-

ing sequence of events: Two weeks after implantation the deposit appeared as a shadow, 3 by 5 mm. in diameter, consisting of coarsely granular material (Fig. 7). The appearance of the shadow did not change materially in the succeeding 4 weeks but at 2 months there was dispersion of granular material over an area that now measured 12 by 4 mm. The shadow consisted primarily of a scattering of small and coarse granules (Fig. 8). Eight months after implantation the shadow began to assume its final triangular appearance and it showed a concentration of granules primarily in the upper portion, whereas the remainder of the deposit showed an opaque shadow (Fig. 9). In the final picture, which was taken post mortem and therefore revealed the details most clearly, one still saw the greatest concentration of metal fragments in the upper portion. However, others were found scattered about in the rest of the calcified lesion, which otherwise showed pronounced mottling. It is evident from this final roentgenogram that the line of demarcation was quite sharp and continuous (Fig. 6). The even greater degree of calcification in the implantation site of another animal is seen in Figure 10. This was observed 1048 days after implantation.

Implantation Sites of Rats

The implantation sites of rats presented a gross appearance similar to those of the rabbits, although they differed considerably in size, as pointed out earlier. Again the lack of a more widespread tissue reaction was striking. As an example, the deposit in the rat that later developed an osteogenic sarcoma of the spine is described in the following: Firmly attached to and partially embedded in the abdominal muscle was a lesion consisting of three components. There was a central oval portion, gray-green with a few black granules visible through the surface. On each end of this oval structure there was a pale yellow focus of firm and seemingly calcified material, measuring 2 and 1 mm., respectively. The central portion measured 3 by 3 mm. (Fig. 13). The roentgenograms of this lesion showed the central portion to contain the fragments of the metal, whereas the rest of the lesion showed a uniformly pale shadow of calcified material (Fig. 14).

Ten months following implantation this animal developed a flaccid paralysis of both hind legs. No evidence of a tumor could be found roentgenographically at this time, but in a film taken 2 weeks later distinct erosion of the left lateral margin of the third lumbar vertebra could be demonstrated. This was associated with some radiolucency of the fourth lumbar vertebra and with loss of the fine bony structure in the bodies of both vertebrae (Fig. 11). No other lesions were seen

in the skeleton. The general condition of the animal became progressively worse, and it was sacrificed 4 weeks later on the 356th day of the experiment. Clinically there had been extreme paralysis of both hind legs and a tumor was palpable in the lumbar spine. Necropsy revealed an extensive tumor involving practically the whole central portion of the lumbar spine. A sagittal section of the tumor showed firm, bone-containing, grayish white tissue, measuring 2.5 by 1.3 cm. There was considerable hemorrhage around the tumor. The terminal roentgenogram showed an extensive osteogenic sarcoma involving primarily the third lumbar vertebra, but also extending into the second and fourth lumbar vertebrae (Fig. 12). Again no other skeletal lesions were seen. There were no metastases. Histologically the tumor proved to be a typical osteogenic sarcoma (Fig. 15).

Radiation Dosage

The radiation dose received by the skeleton of the rat with the bone tumor was calculated in the following manner. The concentration of plutonium found in the skeleton, *i.e.*, the specific activity, was used to estimate the radiation dose received by each gram of bone tissue over a period of some months. The approximate dosage rate formula[7]

$$\text{rep/day} = 60\ CE \qquad (1)$$

was used, where C signifies microcuries, E equals the maximum MEV, and where 60 is a constant. The concentration of Pu in the bones was found to be 0.1 μg./gm. of bone (wet weight). This is equivalent to 0.1×0.063 microcuries * or 0.0063 microcuries of Pu per gm. The maximum energy of the alpha particles of Pu being 5.15, the daily dosage rate to a gram of bone was:

$$\text{rep/day} = 60 \times 0.0063 \times 5.15 \qquad (2)$$

$$\text{rep/day} = \sim 2.0 \qquad (3)$$

If one assumes a silent growth period for the tumor of about 1 month preceding the manifestations of clinical symptoms on the 310th day, and if one assumes furthermore that the concentration of plutonium in the skeleton had not reached the level of 0.1 μg. that was found at necropsy for about 30 days following implantation, a period of approximately 250 days may be considered a likely exposure time of the skeleton prior to the onset of tumor growth. On this basis therefore:

$$\text{rep/250 days} = 2.0 \times 250 = 500 \qquad (4)$$

Since Pu is not distributed evenly in bone or in any other tissue, it is customary to assume a further concentration factor of about 10. On this basis then, the radiation dosage to some parts of the skeleton may, in fact, have been 5000 rep/250 days instead of 500 rep. This

* One microgram of Pu equals 0.063 microcuries.

calculation does not include a factor for the relative biologic effectiveness of the plutonium alpha particles as compared with x and gamma rays. Evans[8] assumed a factor of 5 in the case of alpha particles from radium, but in the absence of experimental data it is impossible to give a specific factor that might reasonably be applied to plutonium. At any rate, it is evident that 5000 rep would be well within the range of radiation sufficient to induce sarcoma in bone; indeed, it probably exceeds it considerably.

Discussion

It is apparent from these observations that a small piece of oxide-coated plutonium metal quickly disintegrates into many fragments once it is in contact with the fluids of the body. This was shown in the animal that died early in the course of the experiment and it was also evident from the roentgenographs of all other animals. It is equally apparent that despite this fragmentation only a very small fraction of the material was actually absorbed from the site and distributed throughout the body. In general, rabbits absorbed more plutonium than rats. It is uncertain whether this was due in part, at least, to the relatively greater extent of the local tissue reaction surrounding the metal in these animals. Indeed, the finding in one of the rabbits would not be in accordance with such a view, for of all the animals surviving more than 2 days it had the smallest site of implantation and yet it had absorbed the greatest amount of Pu. Rabbits as well as rats can harbor a considerable quantity of plutonium locally in the subcutaneous tissues without showing any evidence of it, either by excretion or by analysis of the tissues, as was shown in the animal that died on the second day following implantation. This, and the exceedingly low urinary and fecal excretion rates in all other animals subsequently, would seem to indicate that absorption of the metal from the site was a very slow process. It is also evident that urinary and fecal excretion of Pu is a measure only of that portion of the plutonium in the body that has been metabolized, for at no time have the excretion values been indicative of the amounts of Pu actually present in the bodies of these animals.

The question arises, therefore, whether it is possible that measurements of excreta of humans similarly will always reflect the true plutonium burden of some exposed individual. This could conceivably be the case in an individual who has inhaled particulate matter either consisting wholly of Pu or partially coated with it. A particle of this type, lodged somewhere in the respiratory tract, may in the course of time and under the proper conditions be the source of serious consequences locally, due to the persistent exposure to alpha rays of the

surrounding connective tissue cells, and give no evidence of its presence by the customary methods of plutonium assay. Although such local lesions have not been observed at the site of implantation in the present series of animals, it has been shown in previous work that plutonium nitrate injected locally under the skin or into a leg muscle of mice and rats has caused malignant neoplasms to develop at these sites.[4,9]

Excretion studies showed no detectable excretion of plutonium in the rabbits whereas small amounts were excreted by the rats. Measurable excretion of plutonium in the rat did not commence until sometime between the 10th and 15th week following implantation. The samples collected at that time showed small amounts, with a ratio of urinary to fecal excretion that was in accord with previous studies on rats[2] and also on dogs.[3] The samples collected several months later showed a significant drop in excretion, which probably indicates that absorption from the site of metal deposition had fallen to a very low rate. It appears unlikely that the failure to demonstrate plutonium in rat excreta during the first 3 months of the experiment was indicative of a complete lack of absorption of Pu during this period. However, the available data furnish no clue to explain the lack of evidence for excretion. This is also true for the rabbits which never showed detectable amounts of Pu in either urine or feces although they had absorbed three times more plutonium than the rats.

The relative concentration of plutonium in the liver and skeleton showed a considerable difference in the two species. The rabbit with high specific activity of Pu in the liver more closely resembles the dog[3] and man[10] in this respect. This is in contrast with the rat in which the concentration was considerably less. The plutonium burden in the rabbit is distributed more evenly by the storage of almost equal amounts in liver and bones, and the rabbit is therefore less likely to exhibit late lesions than the rat. This is the case for two reasons: Firstly, a smaller amount of Pu in the bones essentially means a correspondingly smaller bombardment of radiation-susceptible cells in the component tissues of bone with alpha particles. Secondly, an increased storage of Pu in the liver does not imply a correspondingly increased likelihood or danger that late radiation lesions will develop, for, by being stored in the liver, plutonium is embedded in a tissue that is relatively immune to radiation damage as compared with bone. It is apparent from the foregoing that from a given specific dose the rat is more likely to develop late manifestations of plutonium poisoning arising from the skeleton than the rabbit by virtue of the higher concentration of the

material in its skeletal tissues. This is borne out by the animal that died from a malignant tumor of the spine.

The gross appearance of the implantation sites in all animals suggests that oxide-coated plutonium metal is a relatively inert material. The uniformly small deposits in the rats exhibited little fibrous tissue reaction around them and they were exceedingly well demarcated from the surrounding tissues. The deposits in the rabbits, though generally larger, were quite similar to them in their general characteristics. They, too, were conspicuous by the absence of a more abundant tissue reaction. The gross appearance of the implantation site of the rabbit that died on the second day is further evidence for the observation that plutonium metal *per se* was not an irritant to the tissues since the implantation site was remarkable for the complete absence of a grossly discernible inflammatory reaction with exudate or edema. The failure to demonstrate plutonium in the organs of this animal also indicates that the solubility of the metal in normal tissue fluids was negligible at this time.

Calcification of the implantation sites, although not clearly understood, can be regarded as the manifestation of a common principle. It increased with time in all animals and the degree to which it varied among them has been described and illustrated. Plutonium metal implanted in this manner in the subcutaneous tissues shares this secondary calcifying reaction with the nitrate salt of plutonium in solution when it is injected into the leg muscles of rats, for instance.[9] Pu nitrate deposits of this type tended to remain at the site of injection for years after injection and they appeared then as deposits of heavily calcified, granular, amorphous material, clearly demonstrable roentgenographically. Microscopically, these foci were distinguished by the almost complete absence of an inflammatory reaction, in spite of the fact that they still contained considerable amounts of plutonium. They were often surrounded by a thin and avascular capsule of collagenous connective tissue with only a scattering of lymphocytes. The calcified nature of the débris and the thin capsule probably interfered with further absorption of plutonium from the site. In the absence of histologic studies on the metal implantation sites in the present series of animals it can only be surmised that a similar mechanism was at play in them. Indeed, the decreased rate of urinary and fecal excretion in 2 rats at 10 months following implantation would favor the view that absorption of plutonium was rendered more and more difficult with the increasing calcification of the site of implantation.

Further experimentation would be required to study in detail the

sequences in the interaction of plutonium metal with tissues and to correlate them specifically with excretion rates. For purposes of comparison in a more general toxicologic sense one may cite the rare case of lead poisoning that occurs as the result of absorption of lead from numerous small lead pellets embedded in the tissues. Here, too, the pellets by themselves are comparatively inert locally but the harmful effects are brought about by the slow and continuous release into the organism of soluble lead. It should be mentioned that Schinz and Uehlinger[11] have recorded experiments in which malignant tumors were produced in rabbits by the intramedullary implantation into the femora of small quantities of non-radioactive arsenic, chromium, and cobalt in metallic form. These tumors occurred after latent periods of from 4 to 7 years and they were found in the neighborhood of the implants as well as in distant sites. Chemical analyses of the tissues were not reported. It has thus been demonstrated that small quantities of non-radioactive metals are also capable of eliciting tumor formation, when implanted into the tissues. In the present experiment, however, the sarcoma of the spine was undoubtedly the result of the sustained exposure of bone to the alpha particles of plutonium.

Summary

Plutonium metal, when implanted subcutaneously into rabbits and rats, disintegrated into many small fragments soon after introduction into the tissues. The pieces were found to be relatively inert locally, and only small amounts of plutonium were absorbed from the implants during an observation period extending to 1048 days. The implantation sites became heavily calcified in all animals.

Retention and organ distribution studies gave evidence that rabbits absorbed considerably more plutonium under these conditions than rats. The majority of the animals appeared to suffer no ill effects from the local implants or from the absorbed plutonium. However, one rat died with an osteogenic sarcoma of the spine produced by the deposition of plutonium in the skeletal tissues. The data indicate that both species can harbor considerable quantities of unmetabolized plutonium in the subcutaneous tissues without showing evidence of this in the excreta.

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[*Illustrations follow*]

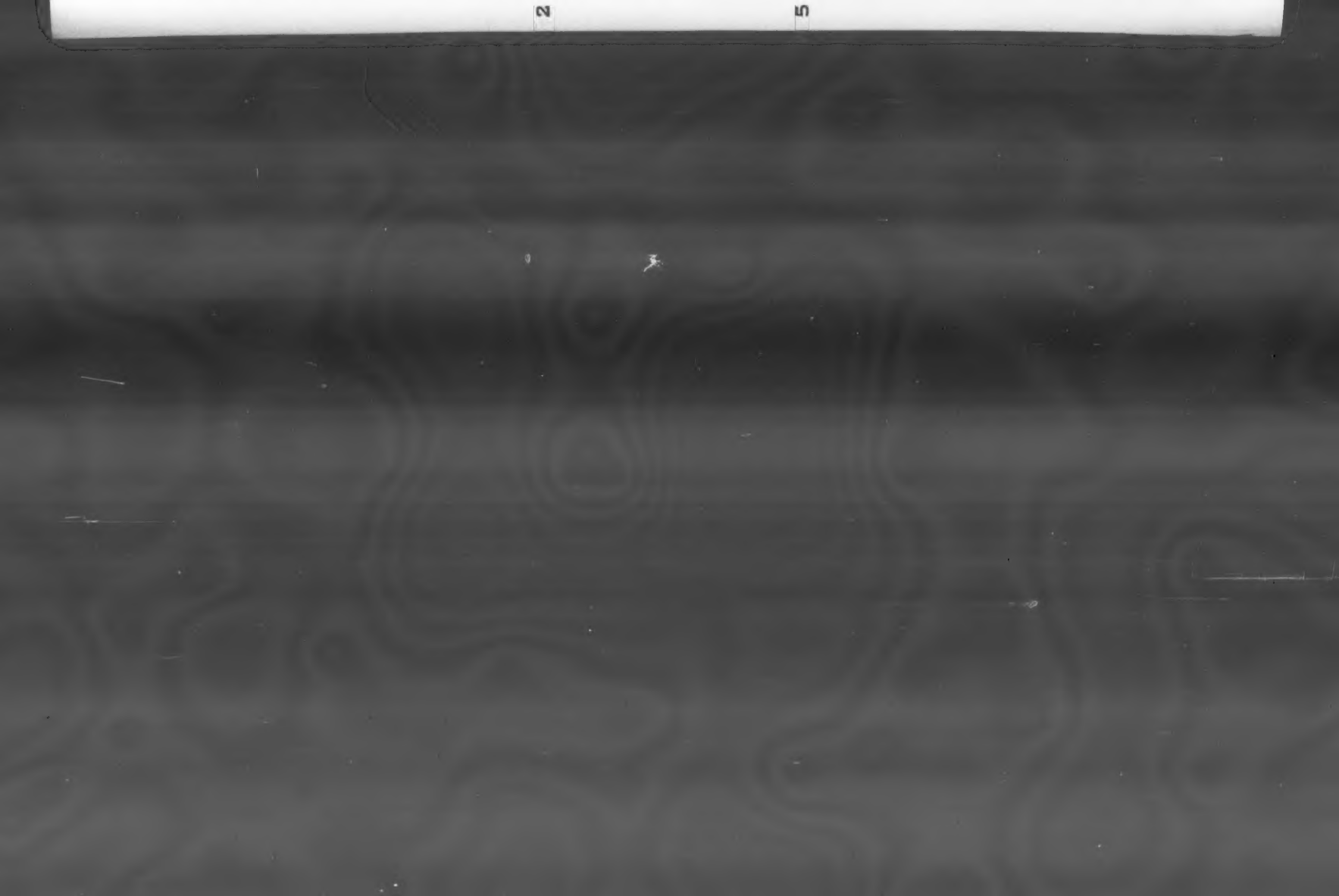
LEGENDS FOR FIGURES

FIGS. 1 and 2. Gross appearance of implant site in a rabbit on second day following implantation, and corresponding roentgenograph.

FIGS. 3 and 4. Gross appearance and roentgenograph of a small implant site of rabbit, 260 days following implantation.

FIGS. 5 and 6. Large implant site of rabbit at 623 days and corresponding roentgenograph.

FIGS. 7 to 9. Consecutive roentgenographs of implantation site of same animal taken during life. For post-mortem appearance see Figure 6.



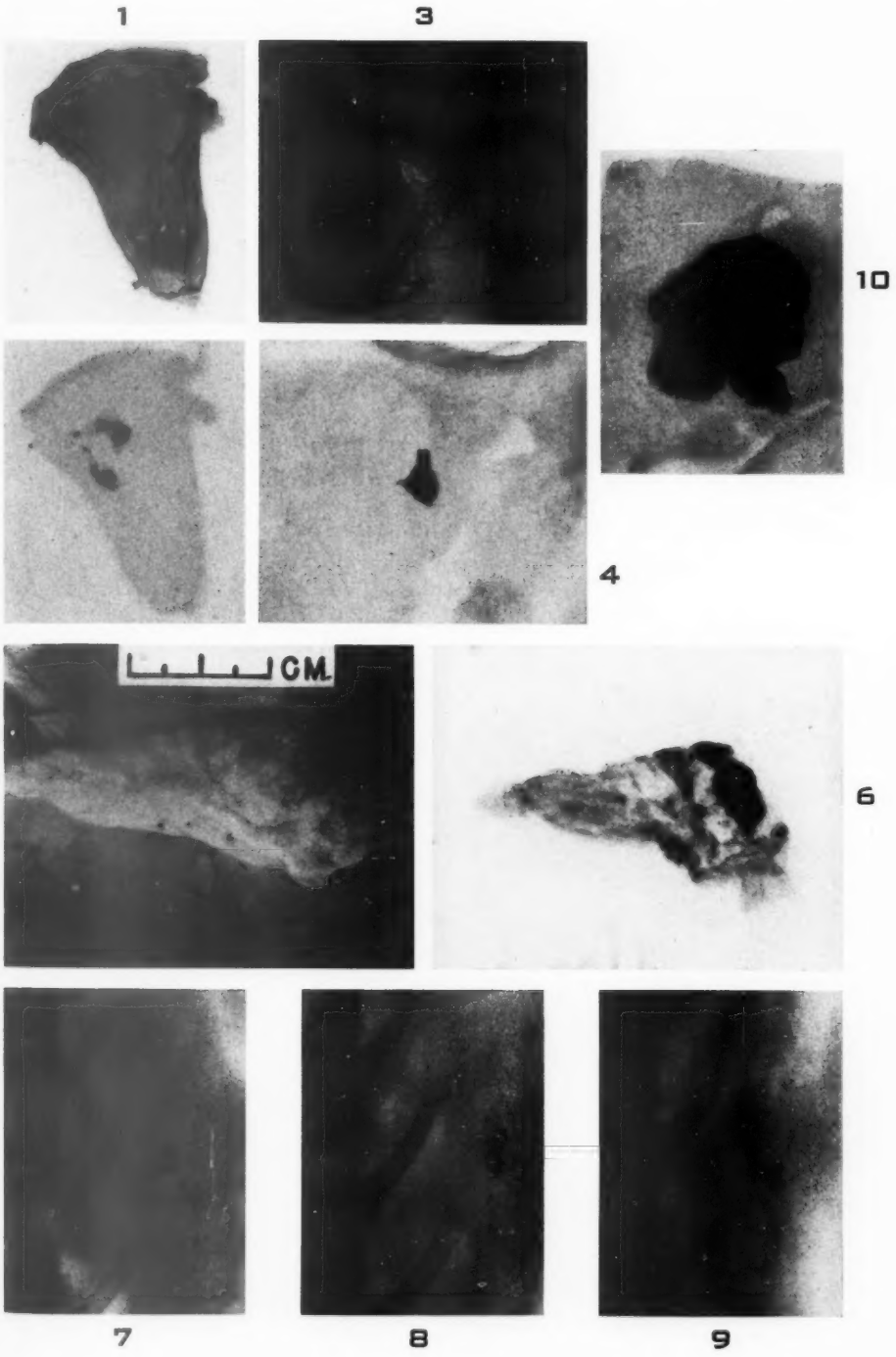

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FIG. 10. Roentgenograph of a large, calcified plaque at implantation site of rabbit at 1048 days.

FIGS. 11 and 12. Roentgenograph of rat with early changes in lumbar spine. Implant of metal on left side (Fig. 11). Osteogenic sarcoma in same area 4 weeks later (Fig. 12).

FIGS. 13 and 14. Implant of same rat at necropsy and corresponding roentgenograph, 356 days after implantation.

FIG. 15. Osteogenic sarcoma of the spine, shown roentgenographically in Figure 12. × 250.

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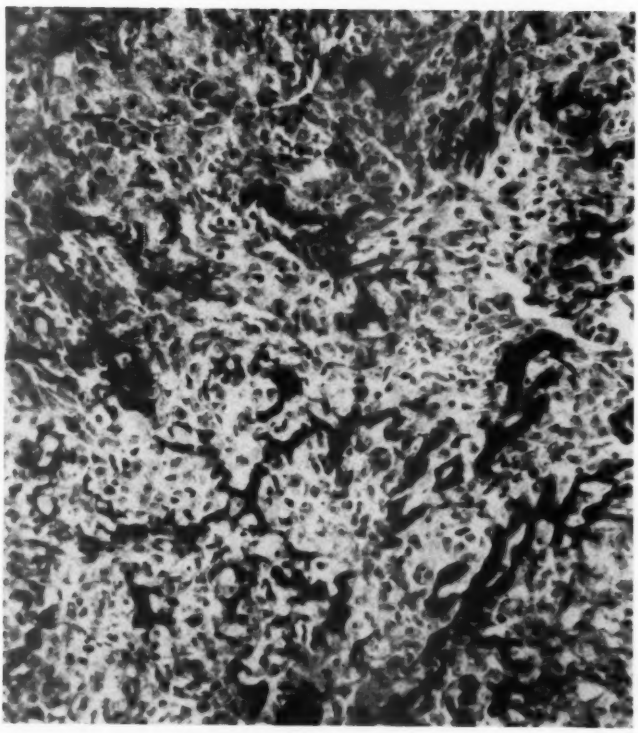

EFFECT OF X-RAYS ON TRICHINA LARVAE *

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In 1921 Schwartz[1] exposed trichinous meat from hogs and guinea-pigs to various doses of x-radiation, measured in arbitrary units † rather than in roentgens. Relatively large doses of x-ray produced no visible evidence of injury to the trichina larvae, but when the latter were fed to rats, they were unable to attain structural maturity. With somewhat lower doses of x-ray, the larvae developed into adult forms in the intestines of the host but the adult worms did not attain sexual maturity; the sex cells of the adult worms appeared to be atrophied, there was no evidence of successful copulation, and no new generation of larvae was produced in the muscles of the host. Levin and Evans,[2] in 1942, demonstrated that trichina larvae, after exposure *in vitro* to x-radiation of approximately 3500 r., were able to undergo maturation to the intestinal phase; that the adult forms copulated, but that no embryos appeared within the mother worms. Alicata and Burr,[3] in 1949, exposed trichinous rat meat to 12,000 r. of gamma radiation of radioactive cobalt (2000 r. daily for 6 days) and on feeding the irradiated larvae to previously uninfected rats, found that sterility was produced in 60 to 100 per cent of the female intestinal worms.

Preliminary to the study of the effect of x-radiation on trichina larvae in pork, it was thought desirable to determine the effect of x-rays on trichina larvae *in vitro*. Specifically, it was desired to learn (1) the dose of x-radiation that would prevent reproduction of trichinae (sterilizing dose) in the white rat; (2) the dose that would prevent development of larvae to adult trichinae (maturation-inhibiting dose); and (3) the dose that would kill the larvae (lethal dose).

The sterilizing dose was then determined for trichina larvae present in experimentally infected rat muscle.

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† According to Schwartz, 1000 units represented the dose of x-radiation given at 5 inches from the focal spot of a Coolidge tube with 4.2 ma., 70 kv. for 24 minutes, or 172 ma. minutes with 6½ inch gap and 5 inch distance.

Method

Trichina larvae were obtained by digestion of muscle of white rats that had been infected 2 to 4 months previously. The trichinized rat carcass, after removal of the head, tail, and feet, was skinned and eviscerated, then macerated in a Waring blender for 45 seconds. The macerated material was cleaner than that obtained by passing the carcass through a meat grinder, and the subsequent process of digestion was more rapid and the yield of larvae greater. Material was examined for larvae both before and after it was digested. The procedures for digestion of the meat and preparation of clean larvae have been described elsewhere (Gould[4]).

The source of radiation was a Keleket Theron x-ray machine, having a General Electric XPT oil-cooled Coolidge tube operated at 200 kv., 20 to 25 ma., with inherent filtration of about 0.15 mm. Cu. In all experiments a Victoreen rate meter was used to indicate the x-ray output, which was held constant for any given experiment by varying the emission of the tube filament. The calibration standard was a Victoreen condenser r.-meter. For the high dose rates employed a 2500-r. chamber was used. The average reading of several timed exposures was taken to determine the dose rate. With filtration by 0.5 mm. Cu plus 1 mm. Al, the dose rate was of the order of 1000 r. per minute.

Larvae were kept in tap water before, during, and after irradiation. In order to improve the conditions of irradiation, the larvae were irradiated in small capsules made of lucite and a section of a lusteroid test tube having a diameter of 15 mm. (Fig. 1). The volume of the capsule was about 3 ml. and the larvae were contained in about 2.2 ml. of tap water. The capsule was threaded to a lucite shaft (Fig. 2) which was rotated by a stirrer during irradiation at about 150 r.p.m. The presence of an air bubble in the vial containing the larvae resulted in more thorough stirring of the organisms, thus increasing the uniformity of dose to individual larvae. The dose rate was measured after placing the sensitive tip of the Victoreen condenser probe within a 50 ml. lusteroid tube having an internal diameter of 2.9 cm. (Fig. 3) in order that the center lines of the probe and of the tube to be irradiated could be made congruent. The measurement of roentgen dosage was believed to be accurate within $\pm$ 2 per cent in international roentgens corrected to 0° C. and 760 mm. Hg, and the experimental reproducibility was within 2 per cent.

Viability of larvae irradiated *in vitro* was tested by the following methods:

(1) Examination on a warm-stage incubator (43.3° C., 110° F.)

for evidence of motility. The larvae were classified as coiled or uncoiled, motile or non-motile, and 200 larvae were counted immediately after radiation, after 1 or more hours post-radiation, and after 1 and more days post-radiation. All larvae placed in the incubator were discarded after the counts were made.

(2) Feeding by means of a stomach tube of approximately 5000 larvae to each of a number of previously uninfected mature white rats having a weight that averaged about 250 gm. Several different strains of white rats were used.

(a) Examination of contents of small intestines of one half of the number of rats 6 days after feeding, for presence of adult trichinae. The small gut was cut into segments 15 to 20 cm. long. While the upper end of a segment was held with toothed forceps in the left hand, the portion of the segment immediately below the end was compressed by a pair of small straight-armed forceps held in the right hand and with a series of downward stripping movements the contents of the segment were evacuated into an open container. All of the evacuated contents were then filtered and washed with tap water through a coarse screen (about 18 mesh per inch) and the filtrate was made up to a volume of 500 ml. The residue was examined for the presence of worms. The filtrate containing the bulk of the trichinae was then centrifuged and the sediment diluted to 25 ml. in a graduated cylinder. After careful mixing, 3 to 5 specimens (each of 0.2 ml. volume) were taken from the mid-portion of the container and examined for adult trichinae in an open Petri dish under a dissecting microscope. Based on the number recovered, the total number of trichinae present in the gut was then calculated. A glass plate, horizontally ruled, was placed beneath the Petri dish, to make counting easier and more accurate. No attempt was made to recover all the adult forms, and the contents of the large bowel were not examined.

(b) Thirty days after feeding of rats with larvae, the remaining animals were sacrificed. Microscopic sections were made of tongue, diaphragm, and muscles of one hind extremity as a check on the digestion method for presence and relative numbers of newly developing larvae. The remainder of the eviscerated carcass was digested and the number of larvae present was calculated.

All counting and feeding experiments with irradiated larvae were controlled by carrying out the same procedures with non-irradiated larvae.

In the experiments with trichinous rat muscle, a similar technique of irradiation was applied. A 50 ml. lusteroid tube, having an internal

diameter of 2.9 cm., was filled with infected rat tissue obtained by cutting (with scissors) a portion of the eviscerated skinned carcass. The tube was mechanically rotated during irradiation (Fig. 4). The muscle was then digested and approximately 5000 larvae obtained from the digested muscle were fed to each of a number of previously uninfected white rats. One half of the controls and one half of the infected rats were sacrificed after 6 days and the contents of the small intestine examined for adult trichinae. The other half of the controls and of the infected rats were sacrificed after 30 days; the small intestinal contents were examined for the presence of adult trichinae, microscopic sections were made from tongue, diaphragm, and muscle of a hind extremity for the presence of larvae and active trichinous myositis, and the eviscerated carcass was digested for recovery of larvae. In all, 26 experiments were conducted involving a total of 426 rats.

Results

Effect on Motility of Larvae

The criterion adopted for viability was motility of the larvae at 43.3° C. It was found that in controls, all viable larvae showed motility at this temperature. Some characteristic positions of larvae during active motility, which is stimulated by exposure to a temperature of 43.3° C., are shown in Figure 5.

The above criterion was adopted when it was found that after exposure to x-radiation in doses of 500,000 r. or more, substantial numbers of coiled larvae could not be stimulated to motility by raising the temperature of the water in which they were suspended; and that when larvae exposed *in vitro* to doses of 50,000 r. or more were fed to rats, no larvae were able to mature to intestinal forms and no reproduction of trichinae took place. Therefore, the test condition that coiled larvae at room temperature are viable and uncoiled larvae are not, does not apply to larvae killed by radiation. However, the criterion that uncoiled larvae were dead or dying still is valid. Five larvae in Figure 5 have assumed the characteristic appearance of uncoiled larvae; the characteristic appearance of coiled larvae is shown in Figure 6.

Results of representative experiments on the effect of various doses of filtered x-radiation on the motility of larvae at several designated intervals of time following irradiation are shown in Table I. It is seen that in two tests, within 2 hours after completion of irradiation with 750,000 r., none or only about 5 per cent of the larvae were still motile. In addition to some variation in response of different batches of larvae to the same dose, with the other doses applied there was a

tendency toward loss of motility at various intervals following irradiation.

Generally, the majority of non-irradiated larvae when kept in tap water at room temperature lost their motility after the fourth day; when kept in the ice box, they retained their motility for 3 weeks or longer. The lethal temperature for trichina larvae is 55° C. (131° F.).[5] When non-irradiated larvae (in experiment 24) were kept at room tem-

TABLE I

Effect of X-radiation in Vitro on Motility of Trichina Larvae

Experiment no.	Dose rate	Duration of irradiation			Total dose	Per cent of larvae motile at designated interval after irradiation			
	r./min.	hrs.	min.	sec.	r.	10 min.	1–2 hrs.	18-24 hrs.	40-48 hrs.
15	0				0 (control)	97.0		91.0	82.0
		1	51	17	100,000	97.0	83.5	75.0	83.5
	899	4	38	13	250,000	72.5	64.5	66.0	53.0
		5	11	45	380,135	65.0	68.5	45.5	35.5
16	0				0 (control)	100.0	100.0	96.0	94.5
		1	37	5	100,000	100.0	98.5	96.5	86.0
	1030	4	2	44	250,000	95.0	96.0	80.0	80.0
		8	5	28	500,000	94.5	80.5	4.0	0.5
		9	42	33	600,000	83.5	78.0	22.5	1.5
		12	8	12	750,000	83.0	5.5	2.5	0.0
24	0				0 (control)	100.0	96.0*	95.0	91.0
		1	37	5	100,000	99.0	98.0	92.5	69.5
	1030	4	2	44	250,000	99.0	94.0	93.0	66.5
		8	5	28	500,000	94.5	93.0	81.5	59.0
		12	8	12	750,000	0.0	0.0	0.0	0.0

* After 12 hours.

perature or in the refrigerator (at 4° C., 39.2° F.) for 1 and 2 days, and then exposed to a temperature of 48.9° C. (120° F.) for a few minutes, there was no appreciable number that showed loss of motility; when exposed to a temperature of 57.2° C. (135° F.) all of them lost their motility. Larvae exposed to x-radiation in doses of 100,000 and 250,000 r. (Table I, experiment 24) did not show evidence of loss of motility after being subjected to a temperature of 48.9° C. (120° F.); but those that had been exposed to a dose of 500,000 r. showed 59 per cent motile forms at a temperature of 43.3° C. (110° F.) and only 21 per cent of motile forms at a temperature of 48.9° C. (120° F.).

No striking difference in the percentage of motile forms was found as a result of exposing larvae to unfiltered x-radiation in any of the following media: (1) buffered solution of potassium acid phosphate and di-sodium phosphate, (2) saline solution, (3) distilled water, and (4) tap water. The doses of irradiation applied in each of these media were

5000, 10,000, 100,000, 250,000, 500,000, 750,000, and 1,000,000 r.

No effect on motility of non-irradiated larvae *in vitro* was observed as a result of exposure to a strong light (100 watt, 120 volt Mazda light bulb) at a distance of 4 inches for a period of 6 hours.

Effect on Ability of Larvae to Mature to Adults and to Reproduce

In Table II are listed the results of two representative experiments on the effect of different doses of x-radiation on the ability of larvae to mature to adult forms and to reproduce. Four or 6 rats were each

TABLE II

Effect of X-radiation in Vitro on Ability of Trichina Larvae to Mature to Adult Forms and to Reproduce

Experiment no.	Dose of x-ray (r.)	No. of rats, each fed 5000 larvae	Adult trichinae recovered from gut after 6 days*		Larvae recovered from muscles after 30 days*	
13	0 (control)	4	2250	875	62,500	75,000
	3,500	4	1850	750	2	3
	4,000	4	75	35	0	0
	4,500	4	161	74	0	0
14	0 (control)	4	1250	1750	49,000	62,500
	3,500	4	1100	85	63	29
	4,000	4	0	38	7	0
	4,500	4	2	0	0	89
	5,000	4	3	0	0	0
	10,000	4	0	0	0	0
	25,000	4	0	0	0	0
	50,000	4	0	0	0	0

* Each number represents the finding in a single rat.

fed approximately 5000 larvae that had been exposed to the specified doses of x-ray. One half of all the experimental rats were sacrificed 6 days after they were fed larvae, and the contents of their small intestine were examined for adult worms. In general, a dose of 4000 to 6000 r. resulted in a marked reduction or in complete prevention of development of the intestinal forms. In an experiment (no. 9) not shown in Table II, however, two adult worms developed from larvae exposed to 10,000 r. unfiltered x-ray and in another rat two intestinal forms developed from larvae irradiated with 30,000 r. unfiltered x-ray.

In experiment 7, larvae that had been exposed to unfiltered radiation in doses ranging from 400 to 5000 r. were found to be infective even when fed to rats 7 days after irradiation. At the time of feeding, 21 per cent of non-irradiated larvae and from 18 to 49 per cent of irradiated larvae were motile. Small numbers of adult forms were recovered from the gut of control and test rats; larval forms were recovered from the muscles on digestion and were seen in microscopic sections from control rats and from those fed with larvae irradiated with doses

up to 4000 r. inclusive, but not with doses of 4250, 4500, 4750, or 5000 r.

In several experiments, it was surprising to recover from the gut, 6 days after tube feeding, larvae that had undergone little or no maturation. Thus in experiment 6, 4 control rats were each tube-fed 5000 non-irradiated larvae, 92 per cent of which were motile. When sacrificed 6 days later, one rat revealed only a single adult trichina in its intestinal contents; one had no adults but had 1174 larvae of which 232 were coiled; one had 136 uncoiled larvae; and the fourth had no adult or larval forms. A test rat fed with 5000 larvae that had been exposed

TABLE III

Effect of X-radiation of Trichina Larvae Present in Meat on Their Ability to Mature and to Reproduce

Experiment no.	Dose of x-ray (r.)	No. of rats	Adult trichinae recovered from gut after 6 days*			Larvae recovered from muscles after 30 days*		Presence of larvae in microscopic section of tongue*	
17	0 (control)	4	3250		1600	185,000	220,000	++++	++++
	5,000	3	250		150	6,250		+	+
	10,000	4	1035		300	1	12	0	0
18	0 (control)	4	210		442	200,000	432,000	++	++++
	10,000	6	130	0	100	1 0	0	0 0	0
	15,000	6	0	0	0	0 0	0	0 0	0
19	0 (control)	4	900		750	475,000	(died, 19 days)	++++	++++
	10,000	6	625	480	262	2 1	0	0 0	0
	15,000	6	0	0	0	0 3	1	0 0	0
22	0 (control)	4	2400	2200		50,000	37,500	++	++
	10,000	6	0	137	0	23 0	0	0 0	0
	12,500	6	3	0	32	1 0	0	0 0	0
	15,000	6	0	0	0	4 0	10	0 0	0

* Each number represents the finding in a single rat.

0 indicates no larvae seen in section; +, an average of less than 1 larva per low-power field; ++, 1 to 4 larvae per low-power field; +++, 5 to 14 larvae; and ++++, 15 or more larvae per low-power field.

to 250,000 r. was found after 6 days to have no adult forms but had 6 larvae. All of the rats in this experiment, however, harbored tapeworms (*Hymenolepis nana*), varying from a few to about 200 in number.

In experiment 8, a control rat sacrificed 9 days after being fed 5000 non-irradiated larvae was found to have in its small bowel 376 adult trichinae and in addition 34 larvae, 10 of which were coiled and motile at room temperature.

In Table III are listed the results of several experiments in which trichinous rat meat was exposed to x-rays in a tube having an internal radius of 1.45 cm. A dose of 12,500 r. largely prevented maturation of larvae to adult forms; after a dose of 15,000 r. no adult forms were

recovered from the small intestine in any of 9 rats sacrificed 6 days after feeding; however, 4 of 9 rats revealed small numbers of muscle larvae (from 1 to 10), 30 days after feeding. Thus, as in the case of irradiation of isolated larvae *in vitro,* when trichinous meat is irradiated a larger dose of x-ray is required to prevent maturation of larvae to adult forms than is required to prevent reproduction. In irradiation, both of isolated larvae and of trichinous meat, relatively few larvae will be resistant to the usual sterilizing and maturation-inhibiting doses.

Microscopic sections revealed a mild degree of trichinous myositis in each of 2 rats fed with 5000 larvae obtained from meat irradiated with 5000 r. No muscle trichinae were seen in microscopic sections of tongue, diaphragm, or muscle of an extremity of any of 11 rats each fed with 5000 larvae obtained from meat irradiated with 10,000 r.; from any of 3 rats each fed with 5000 larvae from meat irradiated with 12,500 r.; or from any of 9 rats each fed with 5000 larvae obtained from meat irradiated with 15,000 r. In one section of skeletal muscle from one rat fed with larvae from meat irradiated with 10,000 r., an area of focal myositis, compatible with that caused by trichinosis, was found. It is obvious that while the technique of microscopic section serves as a check on the accuracy of the digestion method of recovering larvae, it is not as delicate a method for recovery of larvae, particularly from muscle containing small numbers of larvae.

In practically every instance, the finding in microscopic sections of young larvae undergoing encystment in skeletal muscle closely paralleled the findings on digestion. Without exception, when no larvae were recovered by digestion, none were found in the microscopic sections of the particular animal. When a relatively small number (up to about 100) of larvae were recovered by digestion of the entire carcass, the microscopic sections usually revealed no larvae and only occasionally focal areas of myositis compatible with that found in trichinosis.

When the number of larvae recovered by digestion was great (up to 600,000), a corresponding concentration of larvae encysting in the muscles was found. Of the three sites from which microscopic sections were made, usually the lowest concentration of larvae was found in skeletal muscle of a hind extremity, and the highest in diaphragm. Because of the necessity of cutting the thin diaphragm in cross section, however, the tongue was found to be the most suitable of these three sites for microscopic section. In Table IV is shown a comparison of average number of encysting larvae per low-power field of a microscopic section of tongue with average number of larvae recovered from muscles by digestion, per gram of body weight.

Discussion

Reference to Table I shows that with doses of x-radiation over 100,000 r., larvae, in increasing percentage, lost their motility with the passage of time following radiation during the 2-day period of observation, compared with non-irradiated larvae. In addition, with increasing doses of x-radiation, there was an increasing percentage of larvae that lost their motility. Stated in another way, it is evident that doses of 250,000 r. to 750,000 r. injure trichina larvae so

Table IV

Comparison of Concentration of Trichina Larvae in Single Microscopic Section of Tongue with Number of Larvae Recovered by Digestion from Muscles of White Rat

No. of rats examined	Average number of cysts per low-power field in section of tongue*	Average number of larvae recovered by digestion	
		Per gram of macerated carcass†	Per gram of body weight†
21	Less than 1	36	18
15	1–4	360	180
16	5–14	1418	709
8	More than 14	3476	1738

* Diameter of low-power field was approximately 1.3 mm., using a magnification of 100 with a 10 × objective and 10 × wide-field binoculars.

† Carcass was macerated after rat was skinned and eviscerated, and head, tail, and feet were removed. The weight of the carcass thus prepared was approximately one half that of the total body weight, and the average number of larvae per gram of body weight was taken as one half of the number recovered per gram of macerated tissue.

that they lose their motility, first, as the dose is increased and second, with the passage of time following irradiation. A dose of 750,000 r. appears to be critical, from 95 to 100 per cent of larvae losing their motility within 1 to 2 hours after exposure to this dose. With the technique of x-radiation that was applied, there appeared to be a progressive degree of injury with the higher doses, the latent period of loss of motility being longest with the lowest of these doses. Even after exposure to 750,000 r., which required several hours, death appeared to be the result of increasing or cumulative injury.

The finding previously mentioned of reduction, among larvae irradiated with 500,000 r., in number of motile forms from 59 per cent of those exposed to a temperature of 43.3° C. (110° F.) to 21 per cent of those exposed to a temperature of 48.9° C. (120° F.), suggests that when larvae are injured by large doses of x-ray they are more susceptible to sub-lethal temperatures. If this is true for elevated temperatures, it may also be true for low temperatures. If this finding is verified, it may possibly have a practical application in exposure of trichinous meat to a combination of x-radiation and elevated or reduced tempera-

tures. Two of the common methods that are now applied in the processing of certain pork products which are to be sold for consumption without cooking are exposure to low temperatures and heating of the product (*e.g.*, ham) so that its center will attain a temperature of at least 137° F.

The finding of larvae in the gut of some experimental rats 6 and even 9 days after tube feeding indicates that not all larvae undergo maturation or die within 3 or 4 days after being introduced into the intestinal tract. The finding of only a single intestinal form in only one of 4 control tube-fed rats that were heavily parasitized with tapeworms and the finding of many larval forms in 2 of the remaining 3 control rats suggest the possibility that heavy infestation with tapeworms may interfere with ability of trichina larvae to develop to mature adults. The recovery of 1174 larvae from the small intestine of a rat 6 days after it was fed 5000 non-irradiated larvae indicates the presence of some factor inhibiting development. It does not seem likely that so many larvae could remain so long in the gut without being swept out or destroyed. It would seem that they must have been attached to the mucosa but that they lay dormant in respect to development, presumably because of some influence exerted by the tapeworms.

Rotation of the larvae within the vial during irradiation was not a factor in causing loss of motility, since rotation of non-irradiated larvae for 24 hours caused no loss of motility.

The theoretic value of determining the dose of x-ray that produces loss of motility, and assumedly death, of trichina larvae *in vitro* is in determining the minimum dose that would be required to kill trichinae in infected pork. It is obvious that in trichinous pork doses well over 1,000,000 r. would be required to kill infective larvae. Such doses clearly would not be practical as a means of controlling trichinosis. On the other hand, control of the disease need not depend on actual killing of the larvae within the meat; all that is needed is that the larvae present within the meat be rendered non-infective. The evidence of previous workers and of the present study indicates that much lower doses, around 5000 r., which are not lethal, are quite effective in preventing reproduction of the organisms. Apparently the larvae are rendered sterile so that the adult worms that develop from them are not able to reproduce. Furthermore, when larvae are exposed *in vitro* to doses of 6000 to 10,000 r., practically all are prevented from maturing to adult forms.

The variation in resistance of different larvae to the effects of x-radiation was noted previously by Schwartz.[1] This variation applies

to all three effects studied by us, being reflected in the effective doses that produce (1) loss of motility of larvae, (2) prevention of maturation of larvae to intestinal forms, and (3) sterilization of larvae (loss of power of reproduction).

In the experiments with trichinous meat rotated in a cylindric lusteroid tube, the radius of the tube was 1.45 cm., which was equivalent to the minimum thickness of the meat that was exposed to the irradiation at any instant. In these experiments the effective dose that prevented reproduction of the larvae (sterilizing dose) was approximately 15,000 r. This suggests that the doses of x-radiation or other ionizing radiation that would be required to sterilize trichina larvae in half of a hog carcass may not be too large, and conceivably may be within practical limits. In further tests, it is planned to subject trichinous hog carcasses to cobalt-60 radiation. Since this radiation is far more penetrating than that obtained from a 200 kv. machine, the dose of radiation throughout the carcass will be fairly uniform.

Conclusions

A dose of 750,000 r. filtered x-ray applied to trichina larvae *in vitro* caused death of all or nearly all larvae within 1 to 2 hours after irradiation.

Reproduction of trichinae was largely prevented by exposing larvae *in vitro* to x-radiation of 3500 r.; maturation to adult forms was largely prevented by exposure to 5000 or 6000 r.

Pieces of trichinous meat in thicknesses of 1.45 cm. were rendered non-infective to experimental rats when the meat was exposed to 15,000 r. This suggests that the dose of x-radiation required to sterilize trichina larvae in commercial quantities of pork may not be excessive.

We acknowledge the assistance of Mildred L. Ransom, David E. Weyant, and Mary MacPhail.

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Addendum

After submitting this paper for publication, an article on the same subject by J. E. Alicata (Effects of roentgen radiation on *Trichinella spiralis. J. Parasitol.,* 1951, 37, 491–501) came to our attention.

LEGENDS FOR FIGURES

FIG. 1. Vial made of a section of a lusteroid test tube, sealed at one end to a base of lucite. The stopper also is made of lucite.

FIG. 2. Lucite base of vial has been drilled and tapped to receive screw-bolt attachment of lucite shaft of rotating device. The vial contains the larvae suspended in water.

FIG. 3. Arrangement for measuring the dose rate of x-ray by means of a Victoreen condenser placed within a 50 ml. lusteroid tube.

FIG. 4. Arrangement for mechanically rotating 50 ml. lusteroid tube containing trichinous meat while latter is being irradiated.

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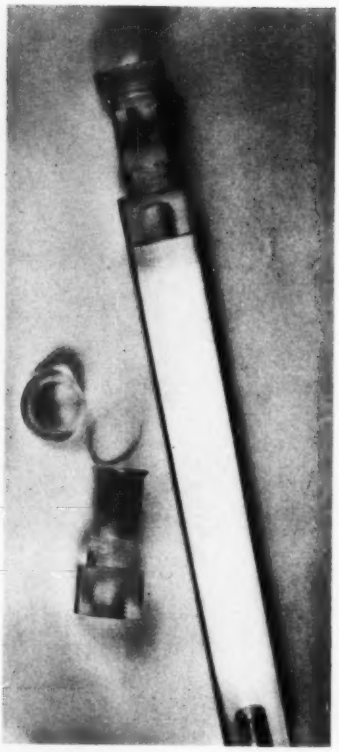

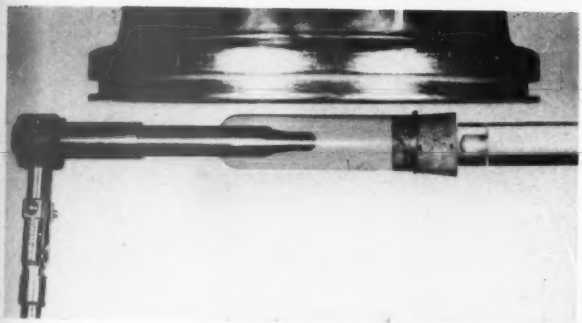

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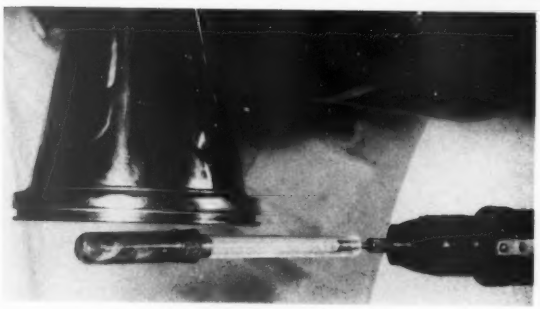

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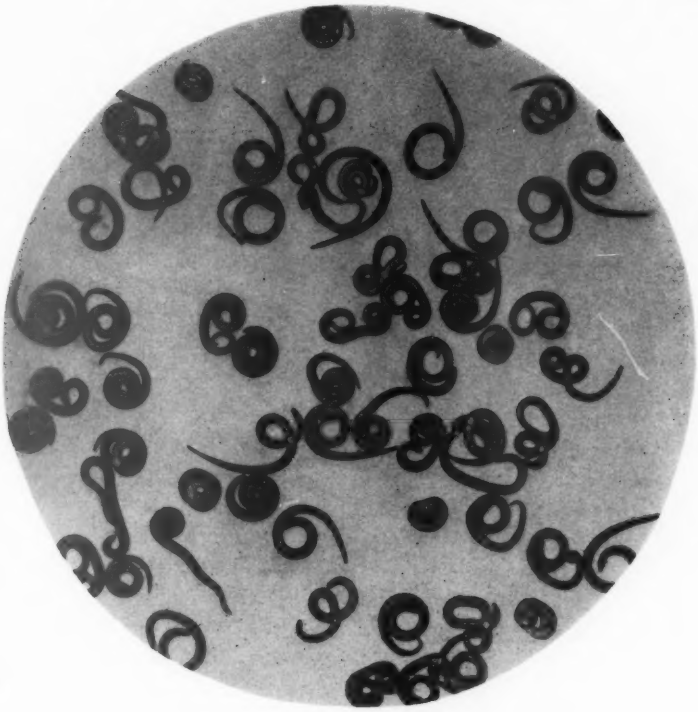
FIG. 5. Shapes assumed by trichina larvae when stimulated to active motility by a temperature of 43.3° C. × 26.

FIG. 6. Four coiled larvae that may be either viable or non-viable. × 86.



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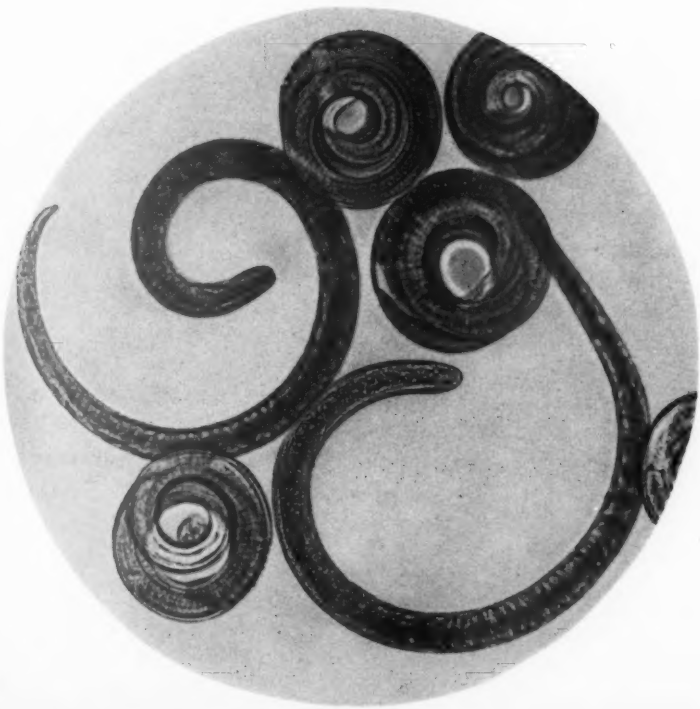

6

BRONCHIAL ADENOMAS ARISING IN MUCOUS GLANDS
ILLUSTRATIVE CASE *

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Bronchial adenoma was first reported in 1882 by Müller[1] as a pathologic entity separate from carcinoma of the lungs. Following this case, some thirty odd cases were reported until 1932, when Wessler and Rabin[2] reviewed the entire picture of bronchial adenomas and attempted to bring some order out of the confusion regarding origin and terminology. They believed that the adenomas of the bronchus originated in the duct epithelium of the mucous glands, and in support of this theory cited the facts that the tumors are invariably covered by an intact epithelium and lie on an uninterrupted basement membrane. This, in their opinion, eliminated bronchial epithelium as the site of origin. The cellular elements of the adenomas did not resemble the cellular elements of mucous glands, and no apparent transition from normal mucous glands to tumor could be seen. By exclusion, however, Wessler and Rabin reasoned that the only possible source was the gland duct epithelium. These authors believed that the term benign bronchial adenoma could adequately designate these tumors, even though there were histologic differences in various cases. This account of Wessler and Rabin marked the beginning of the clinical recognition and treatment of these lesions.

Hamperl[3] and Stout[4] believed that the tumors arose from the peculiar cells known as oncocytes or pyknocytes, which are found in mucous and serous glands and their ducts of adults. These cells are large and contain voluminous acidophilic cytoplasm and deeply stained pyknotic nuclei. The gland-like spaces formed by them do not secrete mucin.

Welt and Weinstein[5] and Edwards and Taylor[6] believed that these tumors were of endothelial origin. Most observers have considered them to be quite similar to the carcinoid tumors of the appendix. In the cases which resemble carcinoid tumors, argentaffin granules have rarely been demonstrated. Only one case reported by Holley[7] and one cited by Foot[8] contained argyrophilic granules.

Womack and Graham[9] postulated that the point of origin was from more than one type of cell in vestigial fetal lung buds. This concept of mixed cell origin, subsequently giving rise to the production of a

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tumor in which bone, cartilage, and various epithelial patterns are found, has not been wholly accepted, although the name is in wide usage. Allen,[10] Kramer and Som,[11] and Brunn and Goldman[12] argued that bone and cartilage formation is caused by dedifferentiation of adult epithelial cells as in adenomatous tumors arising from glands elsewhere in the body.

The widespread acceptance of the belief that these tumors arise from mucous and serous glands is founded on the reasons advanced by Wessler and Rabin,[2] namely, that bronchial adenomas apparently never arise from the smaller bronchioles in which serous and mucous glands are absent. Many of the tumors form definite acini, while others show an obscure alveolar arrangement. The substance found in the lumina generally has not been identified as mucin,[13] but Mallory cited one case in which he stated that the cells did produce mucin.[14] Although the various reasons form a somewhat unstable base for the theory of mucous and serous gland origin, this seems to be the most plausible view at the present time.

The names originally applied to bronchial adenomas were confusing because various pathologists reporting the histologic features of one or several cases drew the erroneous conclusion that *all* bronchial adenomas were of the types they had encountered. Later work with larger series showed varied architecture among the examples encountered and variation of cell forms in a single tumor. Before Wessler and Rabin[2] applied the term adenoma, various types had been called sarcoma of alveolar pattern,[15] cylindrical cell carcinoma,[16] basal cell carcinoma,[17] carcinoid and cylindroma,[3] benign glandular tumors,[18] vascular adenomas,[19] endotheliomas,[5] and mixed tumors.[9] More recently, McDonald,[13] Van Hazel, Holinger, and Jensik,[20] and Huizinga and Iwema[21] proposed a division of adenomas and cylindromas, with the distinction that the adenoma is distinctly benign and the cylindroma potentially malignant. Goldman and Conner[22] suggested a classification based on a decreasing order of malignancy and consisting of mixed tumor (cylindroma), carcinoid, myoblastoma, and benign glandular bronchial tumor.

The concensus of opinion is that the tumors should be termed adenomas with distinction between the (1) carcinoid and (2) the cylindromatous or mixed type.

Gross Description

The gross appearance of bronchial adenomas varies considerably. They may measure from 0.5 to 8 cm. in diameter. When seen bronchoscopically, they appear as pink, purplish, or sometimes pale spheri-

cal or ovoid masses. They may be attached by a broad base, or be distinctly polypoid with a definite pedicle. The surface is either smooth and shiny, or finely granular. Occasional ulcers may appear. By close inspection of the surface, many blood vessels may be seen. This accounts for the great tendency for bleeding to occur with manipulation during bronchoscopy. They are usually hard or firm, but very infrequently may be soft and friable. The size of the endobronchial portion tends to be deceptive, because extensions of the tumor into the bronchial wall and into the adjacent lung tissue beyond may constitute a large part of the tumor mass. The extramural portion is often of greater size than the intraluminal portion. Because of this, bronchial adenoma has been compared by McDonald[13] to an iceberg. Brunn and Goldman[12] illustrated in excellent cross-sectional drawings the endobronchial, intramural, and extrabronchial distribution of the tumors in their cases. Brock[23] cautioned that the expansion of the tumor external to the bronchial wall should not be mistaken for infiltration because epithelial structures, *i.e.*, mucous glands, are normally present outside the cartilaginous rings. The condition of the lung distal to the tumor will depend entirely upon the degree of bronchial obstruction. Emphysema, atelectasis, bronchiectasis, and pneumonitis may be present.

Histologic Findings

Histologically, these tumors have provided ground for much discussion and wide divergence of opinion as to their origin. Most pathologists agree on the type of cell, but there the agreement usually ends. The patterns have been of various types. Arrangement of cells in cords, small gland or gland-like spaces, the presence of bone and cartilage, and the types of secretion have evoked a wide range of opinion concerning their genesis.

In adenomas of the carcinoid type the cells approximate the size and shape of lymphocytes or plasma cells. Comparison has been made also to basal cell carcinoma, islet cell tumors of the pancreas, and parathyroid adenomas.[23] Variation in the size and shape of the cells is not usually marked, although in some cases they may be small cuboidal or tall columnar. The nuclei are round or ovoid, and normochromatic. The cells are arranged in groups or cords in which attempts at acinus formation are seen. In many cases, the cell groups and cords appear to be drawn away from their connective tissue framework. Among the collections of epithelial cells there is a thin stroma of supporting connective tissue which frequently contains vascular spaces lined by endothelium. In this respect morphologic characteristics similar to those of vascular tumors are presented. This group, which

resembles carcinoids of the appendix and ileum, differs in that argentaffin granules usually are not present in the cytoplasm of its cells.[7,8]

The cylindroma or mixed tumor type presents cells which are small and regular, and with indistinct borders. There is slight variability in the shape of their nuclei. There is a tendency for these nuclei to become hyperchromatic. Mitotic figures are rarely found in either this or the carcinoid type. There is more tendency in the cylindroma than in the carcinoid type to form tiny glandular spaces which very often contain secretion. Rosenblum and Klein[24] reported the case of an 11-year-old child with a bronchial tumor which presented a more distinctly glandular pattern. The glands were lined by tall cylindric cells whose cytoplasm was pale and frequently vacuolated. Mucoid material was present in many of the gland spaces. This secretion, which stains faintly with mucicarmine, has been called mucoid, for it apparently is not true mucus. McDonald[13] found that this substance, unlike mucus, stains red with polychrome methylene blue. At times the cylindromatous pattern presents ill defined spaces filled with secretion, and not true lumina. This type of bronchial tumor may resemble the mixed salivary gland tumor known as cylindroma. The stroma tends to be myxomatous in some examples and partially cartilaginous or osseous in others.

In attempting differentiation of these tumors from bronchogenic carcinoma, study of biopsy material is not without shortcomings. Occasionally, such material may not furnish a representative portion. Squamous metaplasia or distortion of the cells may falsely suggest bronchogenic carcinoma. In the differentiation of small-celled carcinoma, the distinction is not always made easily on the basis of cell structure. However, the presence of mitotic figures, the degeneration of the tumor cells, and the stromal infiltration with inflammatory cells usually are evidence of carcinoma.

The benign nature of bronchial adenomas themselves has been repeatedly questioned and in some instances vigorously opposed. Most observers agree that they are locally infiltrative, but that they do not exhibit the usual invasive tendencies of carcinomas. Castleman[25] reported that one case in twenty showed regional lymph node metastasis. Mallory[14] was of the opinion that adenomas should be classed as benign, although a few show regional lymph node involvement, and a very small proportion metastasize to distant points. Womack and Graham[9] believed that the majority of these tumors become malignant and that the identity of the original tumor becomes lost when full blown features of malignancy have developed. In this concept bronchial adenomas follow adenomas of breast, prostate, thyroid gland, and

rectum. Van Hazel *et al.*[20] reported one case with metastases to lymph nodes, liver, and kidney. McDonald[13] believed that the carcinoid type should be considered benign and that the cylindroma, because of its greater infiltrative tendency, should probably be considered potentially malignant. Moersch and McDonald,[26] in a recent series, reported 77 of 86 cases, 90 per cent, to be of the carcinoid type and 9 cases, 10 per cent, of the cylindroma type. Five of these 86 cases had definitely proved metastases in the regional lymph nodes and/or the liver. It was their opinion that adenomas of the cylindroma type possessed the greater malignant possibilities.

Report of Case Illustrative of Origin in Mucous Glands

T. P. S. was a white male, 54 years old, who was admitted to the University Hospital (chart no. 68941) on December 17, 1951. In October, 1951, he had complained of "generalized aching and feeling warm." His temperature was found to be 102° F. He was given aureomycin and returned to his work as a patrolman after 5 days. After 2 days his temperature rose to 104° F. He then complained of urinary frequency and dysuria. These symptoms responded to chloromycetin after several days. He again worked from November 9 to 13, and did not return to his physician. After November 13 the patient was entirely asymptomatic. There was no malaise, fever, cough, urinary symptoms, weight loss, or change in appetite. Roentgenographs done on November 14 and 27 showed infiltration of the right base.

Physical examination showed temperature, 99.2° F.; pulse, 90; respirations, 24; blood pressure, 130/78 mm. of Hg. The only positive findings were dullness and absence of breath sounds in the right lower lung.

Examination of gastric washings was negative for tubercle bacilli. Hemoglobin was 16.6 gm.; sedimentation rate, 10; hematocrit, 49; white blood cells, 9100 per cmm.; with polymorphonuclear leukocytes 69 per cent, lymphocytes 17 per cent, monocytes 0, eosinophils 14 per cent, basophils 0.

After admission to the hospital, roentgenologic examination confirmed previous findings of a lesion of the base of the right lung, interpreted as segmental atelectasis. Bronchoscopic examination revealed fixation and moderate narrowing of the right lower lobe bronchus. A spheroidal, apparently mucosa-covered mass, approximately 4 by 4 mm., was seen. This impinged upon the lumen of the anterior and cardiac basilar branches. The impression was that of a stenosing right lower lobe tumor, either adenoma or carcinoma. The biopsy specimen (no. 75117) showed many mucous glands, covered by ciliated pseudo-stratified columnar epithelium, in a fibrotic stroma in which there was a patchy lymphocytic infiltration. The section made from the aspirated secretions consisted of fibrin, red blood cells, leukocytes, and small groups of exfoliated epithelial cells which showed no evidence of carcinoma.

On December 27 a right posterolateral thoracotomy was done. An atelectatic area was not identified. After segmental resection of the right lower lobe, the patient made an uneventful recovery and was discharged on January 9, 1952.

The specimen comprised the posterior, anterior, and lateral basal segments with the attached right lower lobe bronchus. Projecting into the lumen of the bronchus was a rounded polypoid mass with a very short pedicle. This mass measured 1.3 cm. in diameter. The tumor was covered by intact mucosa. On section, it appeared composed of

many minute cystic areas filled with mucoid material. The lesion did not appear to invade the bronchial wall.

Histologically, the tumor was composed of closely approximated glandular spaces, each of which was filled with mucoid material. The cells lining the spaces were tall, cylindric, and mucus-bearing. The nuclei were situated at the bases of the cells and were generally round or oval. The lesion was well circumscribed. It was internal to the cartilage and was covered by cylindric-celled mucosa. A few inflammatory cells were scattered throughout the sparse stroma. A moderate number of vascular spaces were present, but these were not prominent, as one would expect in the usual bronchial adenoma. When stained with mucicarmine, the mucoid material in the gland spaces had a very faint pinkish tinge. The lining cells stained deep red. The intraluminal mucoid material probably was chemically altered mucoid material that arose in the cells.

This tumor presented none of the cord-like pattern or small gland-like spaces which are commonly seen in the carcinoid or cylindroma types. No bone, cartilage or precartilaginous tissue was present. The glandular structures were large faithful reproductions of the mucous glands present normally in the bronchial wall. The lining cells were tall and regular, in contrast to the somewhat irregular lining cells of the poorly formed acini of the usual cylindroma. The feeble staining with mucicarmine of the substance present in the acinar spaces was of but little help in proving mucous gland origin, even though the cells themselves did stain well and showed true mucus production.

The unusual histologic picture present in this case demonstrates the need for recognition of additional types for the classification of these tumors. It is obviously neither carcinoid nor cylindromatous. Actually it is more "adenomatous" than most bronchial adenomas, and clearly benign.

Summary

After reviewing the theories of origin of bronchial adenomas, their relationship to carcinoma, histology, and nomenclature, a report of a case is presented of which the history and gross appearance were commonplace, but the histologic structure quite unusual. Definite mucous gland reproduction denoted a distinctly benign adenoma having origin in mucous gland.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. **Polypoid adenoma** projecting into the lumen of the right lower bronchus.

1

FIG. 2. Relationship of adenoma to layers of bronchial wall. Hematoxylin and eosin stain. × 50.

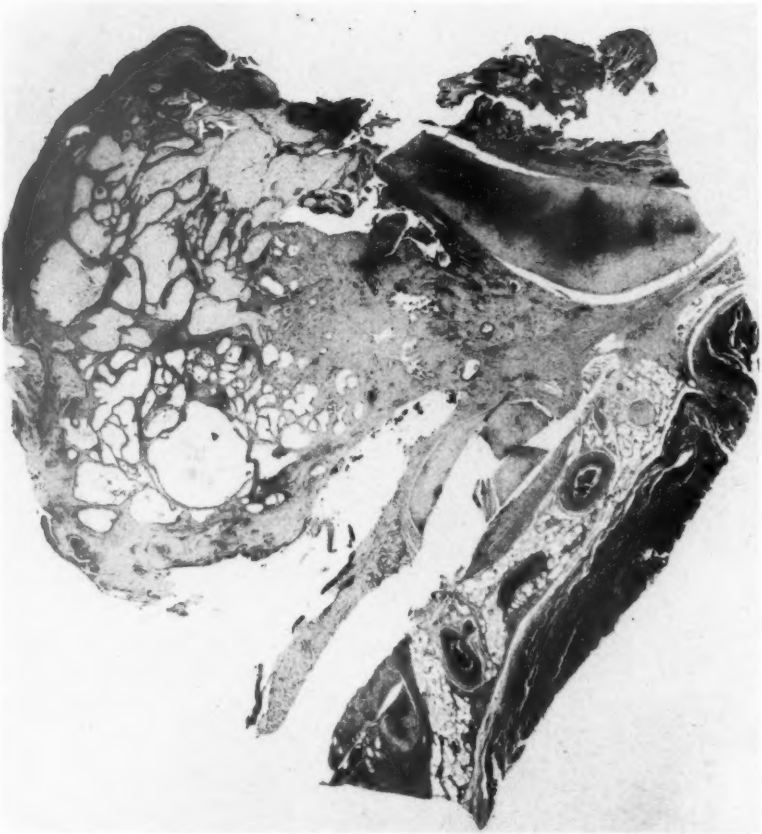

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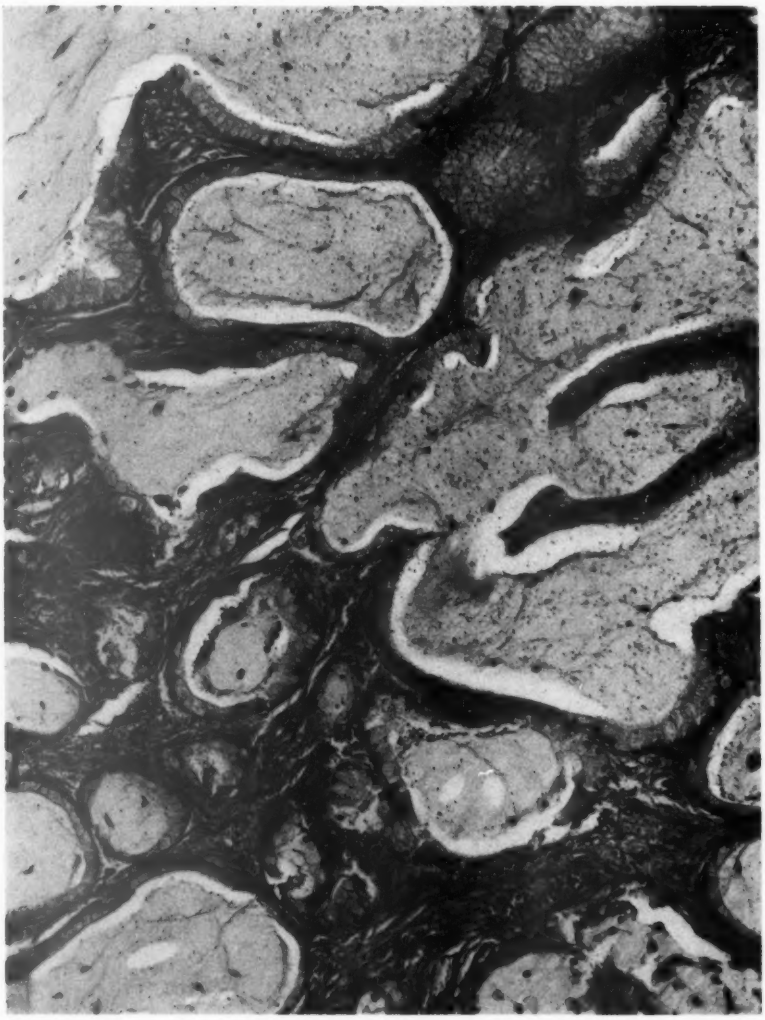
FIG. 3. Photomicrograph showing glandular structure and mucous epithelial lining. Hematoxylin and eosin stain. × 200.



3

ADRENAL CORTICAL ATROPHY IN THE DOG
Report of Three Cases *

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Pathologic alteration of the adrenal cortex morphologically comparable to adrenal cortical atrophy causing some cases of Addison's disease in man apparently occurs rarely as a spontaneous lesion in domestic animals. Reports of such a pathologic entity in the dog were not found in the available literature. Nelson and Woodard[1] mentioned that they too failed to find reference to this lesion in animals. Nieberle and Cohrs[2] stated that no unequivocal case of the counterpart of Addison's disease has been reported in domestic animals. These authors do not, however, specifically mention adrenal cortical atrophy in domestic animals.

Three cases of adrenal cortical atrophy in the dog were encountered at necropsy. This morphologic description is presented because of the apparent rarity of the entity in dogs and because the observations should be of interest to comparative pathologists.

Clinical Features

The dogs were brought to veterinary attention because of varying periods of illness marked by listlessness, anorexia, emesis, and weight loss. No abnormal rectal temperatures were recorded. Hematologic findings in two of the dogs were consistent with extreme hemoconcentration. The blood urea nitrogen of these same dogs was elevated to above 75 mg. per cent. Urinalyses were negative. Unfortunately, no additional laboratory data were determined.

Euthanasia was performed in case of dog A about 25 days after the first reported signs of illness. Dog B died suddenly after an illness of about 1 week's duration. Dog C died about 1 month after the onset of marked clinical disease.

Necropsy Findings

Dog A, a 3-year-old male, was necropsied immediately following euthanasia. The animal was in a fair state of nourishment and showed evidence of marked dehydration. No gross lesions were observed in the pericardium, heart, lungs, thymus, thyroid gland, pancreas, gall-

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bladder, spleen, prostate, urinary bladder, testes, and brain. The liver was dark red and of normal consistency. Its cut surface was somewhat mottled. Several small ulcers were present in the oral mucosa. Small hemorrhagic erosions were found in the gastric mucosa at the pylorus. The small intestine was hyperemic. The kidneys were pale and slightly swollen. Their capsules were not adherent.

Because the adrenal glands were not identified at necropsy, the entire dorsal retroperitoneal area was removed and fixed in 10 per cent formalin. Careful gross serial sectioning later revealed remnants of the adrenal glands. They measured 1.4 by 0.6 cm., were dark brown, and appeared to consist of medullary tissue only. The capsules appeared thickened, smooth, and white.

Microscopic examination of sections of myocardium, lung, gallbladder, pancreas, parathyroid gland, prostate, and small intestine revealed no noteworthy changes. Scattered foci of myeloid metaplasia were found in the spleen and in several lymph nodes. Several small foci of lymphocytes were observed in the thyroid gland. Varying degrees of tubular degeneration were noted in the kidneys. There was considerable centrolobular atrophy and congestion in the liver.

Each adrenal gland exhibited about the same degree of parenchymal destruction and stromal collapse. In most areas the cortical parenchyma was entirely gone and the stroma was condensed to a thin layer. An occasional focus contained vacuolated cells which were believed to be remnants of the zona glomerulosa. There were a few larger masses of eosinophilic cortical cells which showed degenerative changes. Only an occasional area was noted where the hyperchromatic cells suggested attempts at regeneration. Fine granules of yellowish brown pigment were found in some portions of the contracted cortex. The remaining cortical parenchyma and the collapsed reticular stroma were rather densely infiltrated with lymphocytes, plasma cells, and other large mononuclear cells. There was no apparent fibrosis of the cortex with the exception of one small area which resembled a granulomatous process. The medullas were intact.

The anterior lobe of the hypophysis was examined microscopically by median sagittal sections stained with hematoxylin and eosin and by the azocarmine technique. When compared with similarly prepared sections of the hypophysis from normal adult dogs, the anterior lobe showed a definite decrease in acidophil cells. This change was accompanied by a decided increase in the chromophobes. Differential cell counts were not made on any of the sections from this or the subsequent cases.

Post-mortem examination of *dog B,* an 18-months-old ovariectomized female, was completed within 4 hours after death. The animal was well developed and showed no evidence of appreciable weight loss. The mesenteric vessels were markedly congested. The left lobe of the thyroid gland was about one half the size of the normal-appearing right lobe. The tonsils were moderately enlarged. The thymus was prominent and was dotted with petechiae. The pericardium, heart, lungs, mediastinal lymph nodes, pancreas, spleen, gallbladder, kidneys, lower urinary tract, and brain presented no significant gross changes. The liver was of normal size. Its cut surface was mottled with dark red and pale areas. The oral mucosa was bright red and contained several small erosions. The gastric mucosa was reddened. A rather deep ulcer, 1 cm. in diameter, was present in the duodenum near the pyloric ring. Several smaller ulcers were present in the adjacent mucosa, one of which contained a firm blood clot, 1 cm. in diameter. A large amount of tarry, partially digested blood was present in the jejunum. Large, soft, black clots filled the colon. The mesenteric lymph nodes were firm and slightly enlarged.

The adrenal glands were found to be small, dark brown masses embedded in the perirenal fat. The left adrenal gland measured 2 cm. long; the right measured 2.5 cm., was flattened, and appeared to consist of capsule only at one pole. On cross section the capsules were thickened and white. The cortices were not distinctly identifiable since the bulk of the glands consisted of normal-appearing, brown, medullary tissue.

No microscopic changes of note were found in sections of myocardium, kidney, pancreas, stomach, spleen, lung, and thyroid gland. The liver showed marked centrolobular atrophy and congestion. The duodenal ulcer was found to be an acute inflammatory process with necrosis which extended to the serosa, where there was focal peritonitis. The mesenteric lymph nodes and the thymus exhibited lymphocytic hyperplasia and areas of hemorrhage.

The normal architecture of the adrenal cortices was so completely destroyed that in many areas only the capsule and a thin layer of condensed stroma remained. An occasional mass of foamy, degenerating parenchymal cells was observed. Several small nodules of cortical cells were situated within the capsule. The collapsed reticular stroma was rather densely infiltrated with lymphocytes and plasma cells. In the juxtamedullary region the stromal collapse was less advanced so that many dilated, blood-filled vascular channels remained. Small amounts of finely granular yellowish brown pigment were present in the outer

portions of the thinned cortex. The microscopic changes were equally severe in both adrenal cortices. No apparent alteration was observed in the medullas.

Sections of the anterior lobe of the hypophysis were prepared and examined as in the previous case. There was a decrease in acidophil cells and an increase in the chromophobes. Changes in the basophils were not determined in the sections examined.

Dog C, a 5-year-old ovariectomized female, showed evidence of weight loss. No gross lesions were observed in the heart, lungs, liver, pancreas, spleen, stomach, and intestines. The cortices of the kidneys were slightly decreased in thickness. The right adrenal gland was not found. The left adrenal gland was only about one quarter the normal size.

Microscopic examination of sections of pancreas and lymph node revealed no changes. Sections of kidney contained scattered lymphocytic foci in the interstitial tissue of the cortex. Only a portion of a cross section of the left adrenal gland was available for study, but the cortical changes were well demonstrated. The changes resembled those observed in dog A. The cortical parenchyma was completely destroyed and the stroma was contracted to a marked degree. Large vacuolated areas containing finely granular brownish pigment were scattered in the outer zone of the collapsed cortex. In several areas the remaining vascular channels were moderately distended with erythrocytes. Lymphocytes were diffusely infiltrated through the cortical remnant. Occasionally these cells were found in dense masses in the subcapsular zone. The medulla showed autolytic changes only. Only one section of the anterior lobe of the hypophysis, stained with hematoxylin and eosin, was available for microscopic examination. Changes similar to those found in dogs A and B were observed.

Comment

The microscopic features of the adrenal cortices in the three dogs are strikingly similar to those of adrenal cortical contraction in man as described by most pathologists.[3] The cortical destruction appears to be considerably more extensive and severe than that produced experimentally in dogs fed the insecticide, 2,2-*bis* (parachlorophenyl)-1, 1-dichloroethane (TDE).[1]

An increase in the number of chromophobes and a reduction in the number of acidophils and basophils in the anterior hypophysis have been reported in cases of Addison's disease in man.[4] Similar changes were observed in the three dogs in regard to the number of chromophobes and acidophils. This finding is in contrast to that of Nicholson[5]

for adrenalectomized dogs. He reported that no difference could be detected between the anterior hypophyses of adrenalectomized and of normal dogs.

At present the etiology of the spontaneous adrenal cortical changes in the dog appears to be as obscure as it is in man.

Summary

Three cases of spontaneous adrenal cortical atrophy or contraction in the dog are reported. A morphologic resemblance of the lesion to the adrenal changes in some cases of Addison's disease in man is evident. Likewise, the cellular changes in the anterior hypophysis are similar to those which have been described in Addison's disease. This is believed to be the first report of this adrenal lesion in the dog.

I wish to express my gratitude to Dr. Carl F. Schlotthauer, Rochester, Minnesota, for providing me with the data and microscopic preparations from dog C. In addition I wish to thank Dr. J. R. Dawson, Jr., University of Minnesota, Minneapolis, Minn., for his suggestions in the preparation of the manuscript.

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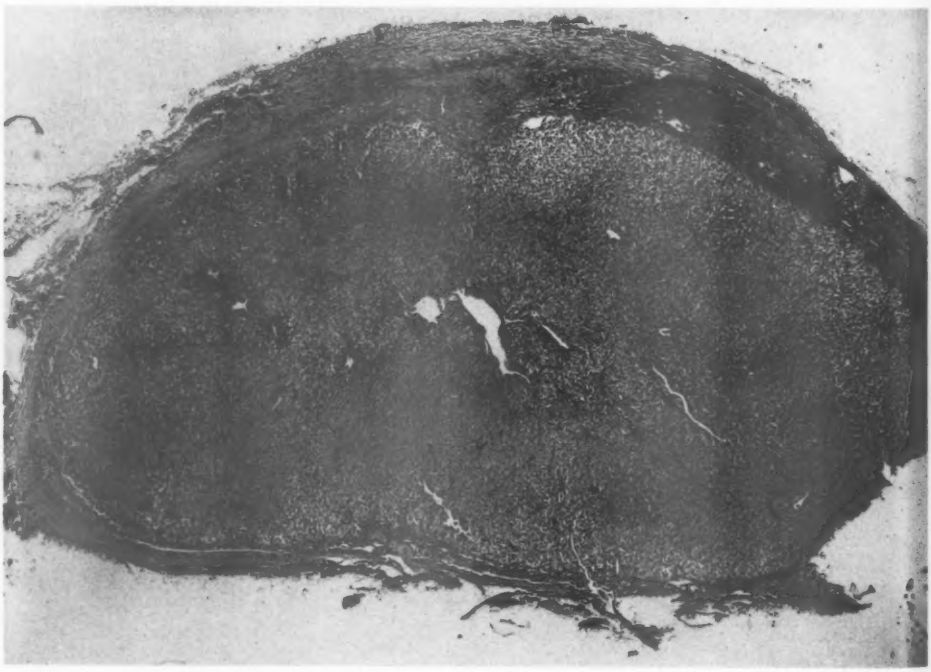
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Dog A. Cross section of an adrenal gland showing extreme cortical destruction and intact medulla. Hematoxylin and eosin stain. × 8.5.

FIG. 2. Dog. A. View of the collapsed adrenal cortex showing lymphocytic infiltration and foamy areas containing the granular material. The normal medulla is below. Hematoxylin and eosin stain. × 112.

FIG. 3. Dog B. Cross section of the left adrenal gland showing the extent of cortical damage. Hematoxylin and eosin stain. × 8.5.



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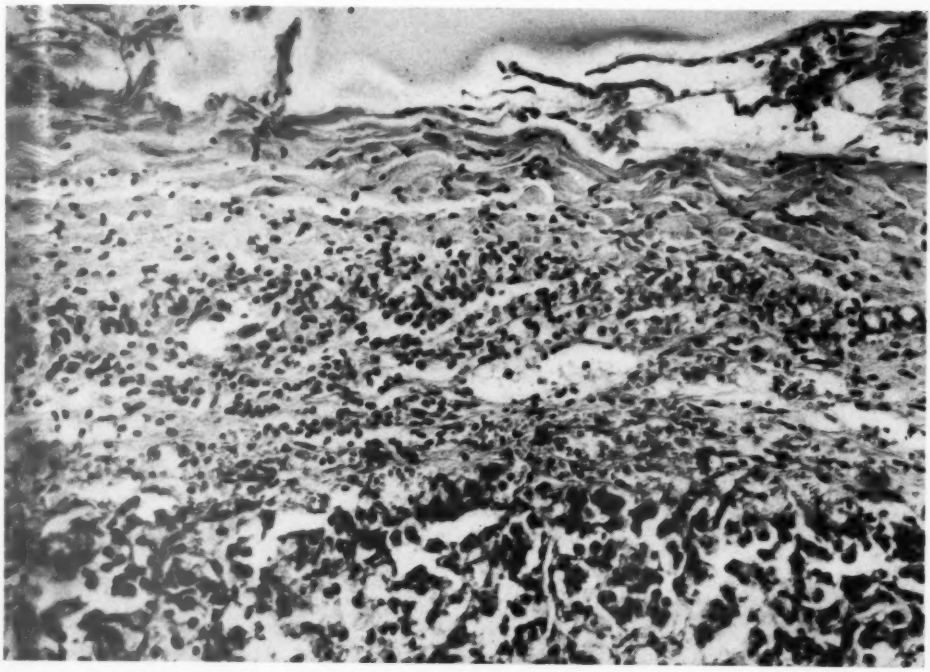

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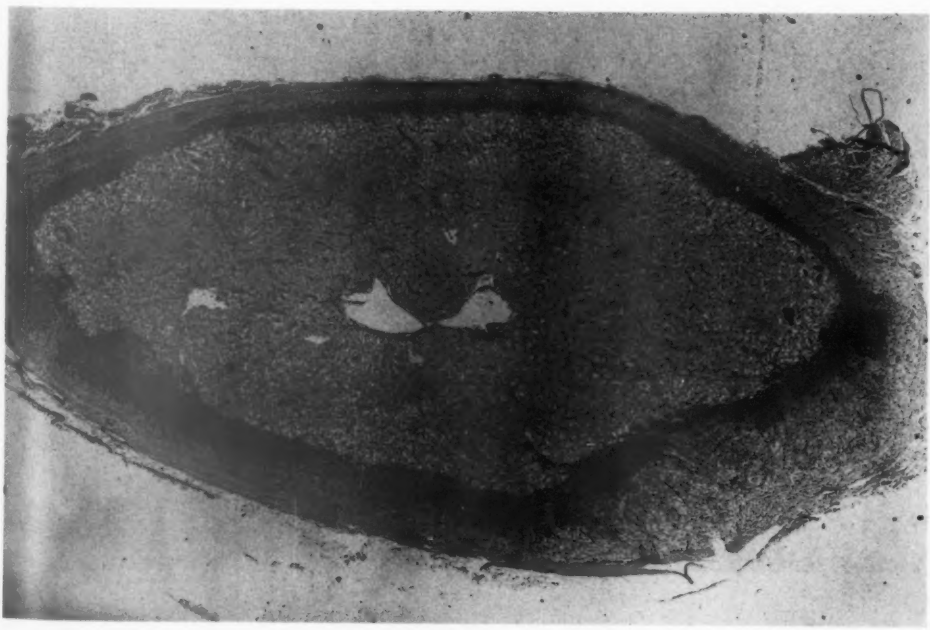

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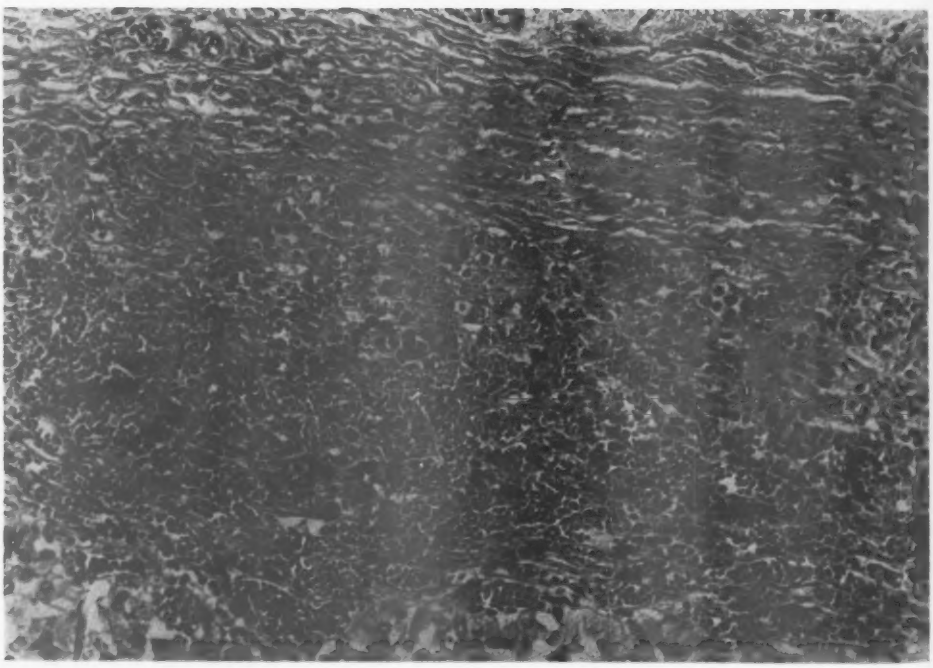
FIG. 4. Dog B. The entire thickness of the remaining adrenal cortex. Of note are the blood filled channels. The intact medulla is the narrow strip below. Hematoxylin and eosin stain. × 112.

FIG. 5. Dog C. Incomplete cross section of the left adrenal gland showing cortical collapse. Hematoxylin and eosin stain. × 12.

FIG. 6. Dog C. Section through the entire thickness of the collapsed cortex. Resemblance to Figure 2 may be noted. The medulla is at the right. Hematoxylin and eosin stain. × 112.



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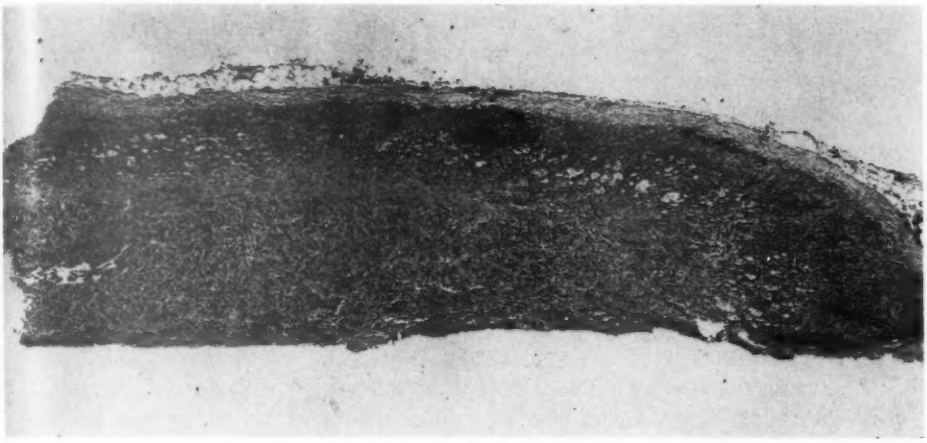

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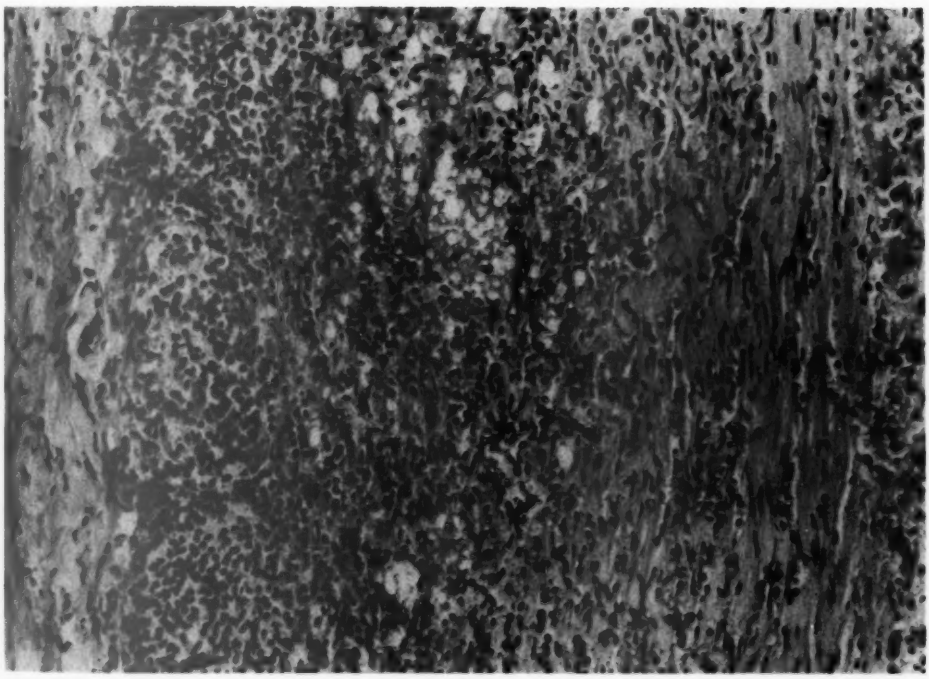

6

HEMOGLOBIN AS A TRACER ANTIGEN IN ANAPHYLAXIS *

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The study of the anaphylactic phenomenon in the various animal species has been greatly facilitated by the use of certain radioactive tracers.[1,2] Warren and Dixon[1,2] in their investigation of the nature of anaphylaxis in the guinea-pig used bovine gamma globulin tagged with I^{131}. Their results appeared to implicate the lungs as the primary site of the anaphylactic reaction.

Studies of iron metabolism being conducted in this laboratory made available a quantity of hemoglobin tagged with radioactive iron. It was believed worth while to carry out a series of experiments designed to determine if this substance might be of value as a tracer antigen. For this purpose hemoglobin appeared to offer certain advantages, among which are ease of labeling and the astonishing rapidity with which extracellular hemoglobin is removed from the blood.[3] This second factor prevents a high level of hemoglobin in the blood, thereby circumventing the error of contamination of tissues.

The studies now being reported had a twofold purpose: first, to decide upon the suitability of hemoglobin as a tracer antigen; and second, to attempt to locate some primary site of anaphylaxis using antigen concentration as the criterion.

PROCEDURE

The hemoglobin used in these studies was prepared in the following manner: Hemoglobin labeled with Fe^{55} † was prepared from the blood of dogs which had been injected with Fe^{55} over a period of 1 year. Non-radioactive hemoglobin was prepared from the blood of normal dogs. The preparation and purification of the hemoglobin solutions were described by Hahn.[4] Two radioactive hemoglobin solutions were prepared. The radioactivity of these solutions were 200 counts per minute per milliliter, and 400 counts per minute per milliliter, respectively. The activity of these solutions was measured according to Hahn.[5] The solution with an activity of 200 cts./min./ml. was used for those animals sensitized and shocked by intracardial injection. The concentration of the hemoglobin solutions in all cases was 25 mg./ml.

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The experimental animals were guinea-pigs, each weighing approximately 450 gm. At the time of the sensitizing injections, these animals were 2 months old.

The guinea-pigs were grouped under four classifications as follows:

Group I. Animals which were sensitized with non-radioactive hemoglobin (I-Hb) and shocked with tagged hemoglobin (Ra-Hb).

Group II. Animals which were sensitized with Ra-Hb and shocked with I-Hb.

Group III. Animals which were sensitized with Ra-Hb and shocked with Ra-Hb.

Group IV. Control animals. (a) Animals which were sensitized with Ra-Hb and sacrificed after the latent period. (b) Non-sensitized animals which were injected with a quantity of Ra-Hb equivalent to a shocking dose and then sacrificed.

Subcutaneous and intracardial routes of injection were used in all groups.

The following optimum levels were established by experiment: (1) Optimum sensitizing dose, 40 to 50 mg. of Hb; (2) Optimum shocking dose, 100 to 125 mg. of Hb; (3) Optimum latent period, 14 to 18 days.

The minimal shocking dose was 100 mg. The volumes of hemoglobin solution were 2 ml. for the sensitizing dose and 4 ml. for the shocking dose.

The organs from these animals were prepared for radioactive assay in this fashion: Following the death of the animals, the organs desired were removed and perfused with saline solution. They were then minced and washed thoroughly. These organs were dry ashed and the ash taken up in concentrated HCl. The residual iron was then processed and electroplated by the method used by Hahn.[5] The activity of the samples was measured by means of a Geiger counter.

Specimens and slides for histologic studies were prepared by the usual methods.

Results

The well known symptoms of anaphylaxis were observed in the guinea-pigs which were sensitized and then given a shocking injection. These symptoms were in this order: sneezing, scratching of nose, respiratory difficulty, bristling of the hair, convulsions and progressive weakening, which sometimes resulted in death depending on the severity of the reaction. The degree of severity was measured on the basis of the duration of the reaction. Those guinea-pigs which did not die from shock but had evident symptoms of the anaphylactic reaction were sacrificed with a sharp blow on the head.

On opening the thoracic cavity of those animals which had been shocked, it was observed that the lungs were enlarged and remained so on exposure to atmospheric pressure. The significance of this observation will be discussed.

A careful scrutiny of the data obtained from the radioactive assay

TABLE I

Experimental Animals: Groups I and III

Route of injection	Group I animal	Liver % uptake	Heart % uptake	Kidneys % uptake	Lungs % uptake	Spleens % uptake	Total % uptake in organs assayed
S*	1	16	3	10	2	0	31
S	2	22	0	31	2	0	55
S	3	16	0	24	0	0	40
S	4	25	5	5	0	0	35
S	5	6	0	0	2	0	8
S	6	65	0	13	5	2	85
S	7	6	0	0	0	0	6
I*	8	15	13	13	33	2	76
I	9	20	10	16	38	2	86
I	10	25	10	15	25	6	81
I	11	6	19	13	16	2	56
	Group III animal						
S	1	25	5	3	6	8	47
S	2	11	10	2	15	6	44
S	3	19	1	15	1	1	37
I	4	20	13	15	30	2	80
I	5	22	15	10	36	5	88
I	6	20	16	12	24	3	75

Group I = Animals which were sensitized with non-radioactive hemoglobin and shocked with tagged hemoglobin.

Group III = Animals which were sensitized with tagged hemoglobin and shocked with tagged hemoglobin.

* S = subcutaneous; I = intracardial.

TABLE II

Control Animals: Group IV, A and B

Route of injection	Animal sub-group A	Liver % uptake	Heart % uptake	Kidneys % uptake	Lungs % uptake	Spleen % uptake
S	1	0	0	0	0	0
S	2	0	0	0	0	0
I	3	0	0	0	0	0
I	4	0	0	0	0	0
	Sub-group B					
S	5	20	0	7	1	0
S	6	25	0	6	0	0
I	7	30	15	6	0	2
I	8	25	25	14	0	7

Group A = Animals which were sensitized with Ra-Hb and sacrificed after latent period.

Group B = Non-sensitized animals which were injected with a quantity of Ra-Hb equivalent to a shocking dose and then sacrificed.

of the organs revealed a significant difference in the uptake of antigen by the lungs depending on the route of injection. It also was seen that anaphylaxis was most severe when the concentration of antigen was greatest within the lungs and the route of injection was intracardial. This difference was not observed to any great extent in the other organs, namely, the liver, spleen, kidneys, and heart. The animals of group IV gave no evidence of any uptake of radioactive hemoglobin. The uptake of antigen is expressed in terms of percentage of the injected dose.

Representative sections of the lungs revealed large areas of acute emphysema as well as intervening areas of edema and recent hemorrhage. Occasional lobules contained coagulated pale pink material in which macrophages laden with dark brown pigment were abundant. In all sections hypercorrugation of the bronchiolar mucosa was obvious. This approached actual occlusion in places, while the associated smooth muscle exhibited many contractile bands. In the liver there was observed a thickening of the blood vessel walls along with an increased affinity of these vessels for eosin. The endothelial cells appeared swollen and in folds in some places. In the kidneys there was an over-all dilatation of the tubules with the exception of the proximal convoluted tubules. There was swelling of the epithelial cells and granulation of the cytoplasm. A feathery precipitate was noticed in the lumina of the tubules.

Discussion and Conclusions

The observed symptoms and the histologic studies indicate quite conclusively that the antigen employed produced anaphylactic shock. The edema in the bronchiolar walls with the resultant decrease in the diameter of the lumina, along with the obstruction produced by the folding of muscular tissue, can account for the emphysema observed on the opening of the thoracic cavities of the shocked animals. The bronchiolar obstruction gave rise to a condition whereby more air was entering the lungs than leaving. This explains the respiratory difficulty. Whether or not the cause of death, in those animals fatally shocked, is primarily respiratory in nature is something that cannot be concluded definitely on the basis of the data collected. It is very possible and somewhat probable that the cause of death is respiratory failure.

An analysis of the data collected by means of radioactive assay reveals that those organs having a significant uptake of antigen are the liver, kidneys, lungs, and heart. The uptake of antigen in the lungs and heart is greater when the route of injection is intracardial, whereas

the uptake of the liver and the kidneys remains approximately the same regardless of the route of injection. It should be noted that the severity of anaphylaxis increases with an increased antigen concentration in the lungs and heart.

The question arose as to why the intracardial injections should bring about an increased uptake of antigen in the lungs and heart and also a more severe anaphylactic reaction. An important factor is the rate at which hemoglobin is removed from the circulation. The animal has two mechanisms by which this hemin protein is removed: by conversion in the liver as the pigment, and by excretion by the kidneys. Due to these factors and especially the rapid renal excretion, it is evident that with subcutaneous injections the slow rate of diffusion of antigen into the blood stream prevents a high level of antigen in the circulation at any given time. It is, therefore, our opinion that the increase of antigen in the lungs when the shocking injection is intracardial is due, in part, to this higher level of antigen concentration in the blood. The increase of antigen in the heart can be attributed most probably to a diffusion of hemoglobin into the tissues.

The kidneys, liver, and spleen are sites of accumulation due to the metabolic and phagocytic action of the liver, the phagocytic action of the spleen, and the excretory function of the kidneys. This accounts for their constant antigen concentration regardless of the route of injection. The antigen uptake of these organs does not change significantly with changes in the severity of the reaction.

The question may then be asked if there is a direct relationship between the antigen uptake of the lungs and the degree of anaphylaxis. There is the possibility that this increased antigen uptake in anaphylaxis is due solely to the route of injection and that the real primary site is located elsewhere. Observations which serve to eliminate this possiblity are, in order of importance: Control animals of the nonsensitized group were given intracardial injection and showed no uptake of antigen in the lungs; the organs were washed free of blood; and the gross symptoms and histologic studies point to an involvement of the lungs.

The liver, heart, kidneys, and spleen are ruled out of intimate involvement in anaphylaxis by having about the same percentage uptake as in the controls.

On the basis of the data accumulated and the observations made, it is believed that the lung in the guinea-pig, using antigen concentration as the criterion, is the primary site of anaphylaxis. Furthermore, these studies indicate that hemoglobin tagged with radioactive iron is well suited for anaphylactic studies.

SUMMARY

Guinea-pigs were sensitized and shocked with dog hemoglobin which had been tagged with radioactive iron.

On analysis of the organs, both by radioassay and histologic means, it was found that the lungs seemed to be intimately concerned with anaphylaxis.

It was concluded that the lungs, on the basis of antigen concentration, were the primary site of anaphylaxis, and that hemoglobin may be a valuable tool in tracer antigen studies.

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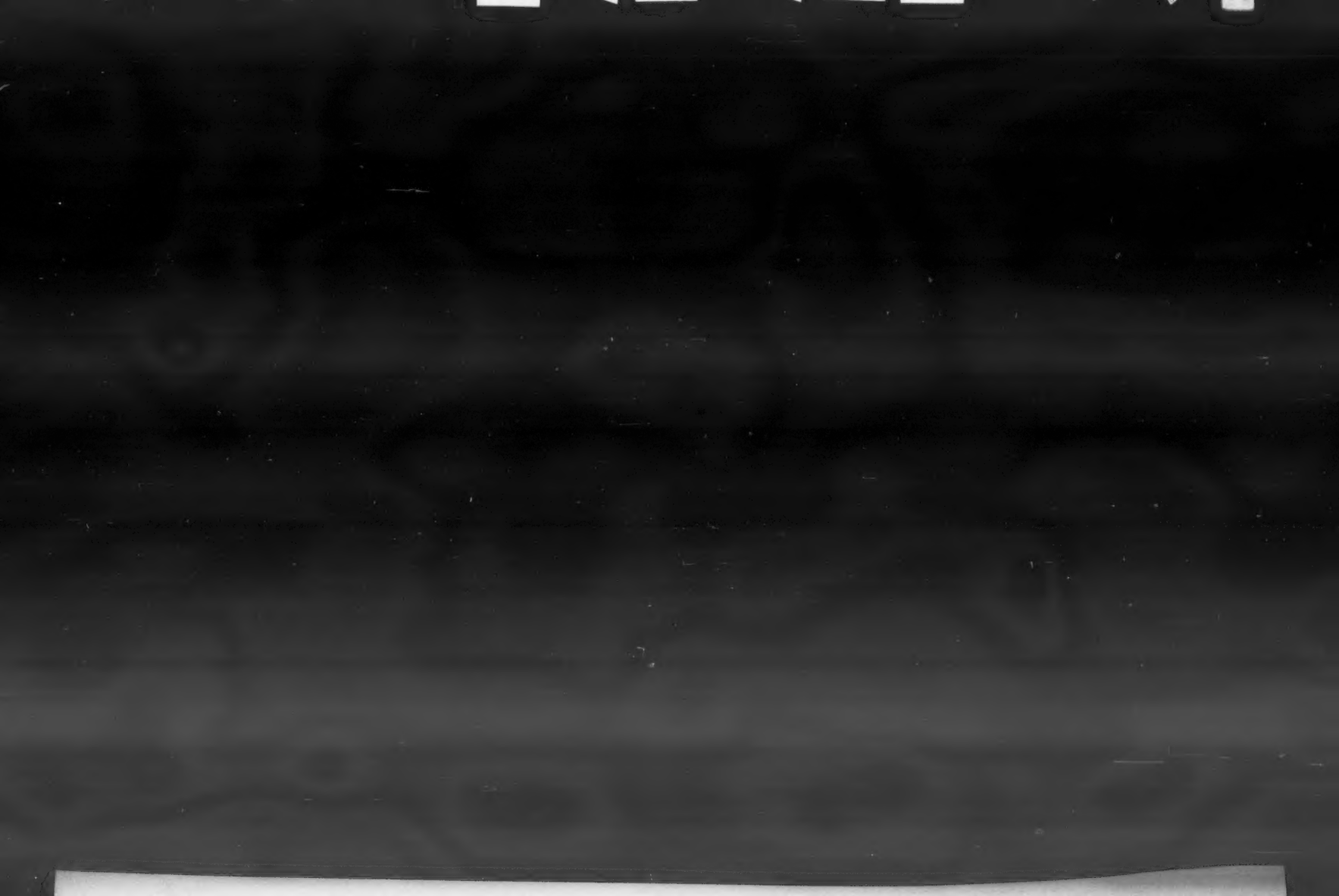
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